ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
VOLUME 82, ART. 2 PAGES 191–644

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CURRENT TRENDS IN RESEARCH AND CLINICAL MANAGEMENT OF DIABETES

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NEW YORK
PUBLISHED BY THE ACADEMY
September 25, 1959

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(Founded in 1817)

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Conference Chairman and Consulting Editor

PETER H. FORSHAM

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* This series of papers is the result of a conference on *Current Trends in Research and Clinical Management of Diabetes* held and supported conjointly by The New York Academy of Sciences, The Upjohn Company, Kalamazoo, Mich., and Farbwerke-Hoechst Ag., Frankfurt (M)-Hoechst, Germany, April 10 and 11, 1959.

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INTRODUCTION

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The collaboration of an active group of investigators to produce this monograph was motivated primarily by the desire to reappraise the status of the ever-accelerating research in the field of diabetes. This was done also in the hope of expediting the clinical application of relevant scientific findings for the benefit of the increasing number of known diabetics.

An acceptable definition of the various types of hyperglycemia has become of increasing practical importance with the inclusion of the sulfonylureas in the therapeutic armamentarium against diabetes. Naunyn already described three different types of diabetes at the turn of the century in his classic text entitled *Der Diabetes Melitus*.[1] This investigator differentiated between: (1) the diabetes of young patients, mostly before the thirtieth to fortieth year, "the pure diabetes"; (2) adult diabetes, mostly "a mild disease of older patients"; and (3) the organic diabetes associated with conditions such as cirrhosis. This classification has taken on practical meaning as the prematurity-onset diabetic will not respond to sulfonylureas, whereas the postmaturity-onset diabetic will usually do so.

The genetics of diabetes is becoming increasingly more important, since we have interfered with natural selection with the advent of insulin and are thereby increasing the reservoir of potential diabetics enormously. We are now capable of preventing the fatal outcome of coma with a high degree of assurance. We can provide patients with at least potentially adequate amounts of insulin on a fairly normal diet, as adjudged by near-normal blood sugar levels much of the time; this may also be done with sulfonylureas and biguanides. However, it has become well recognized that, even with the very best "control," changes in the integrity of the vascular system occur in varying degrees in long-continued diabetes. Among such changes are, notably, the degenerative ones in the small vessels of the eyes and kidneys and the accelerated atherosclerosis of larger vessels. In this monograph contributors discuss progress of the research into this all-important and discouraging phenomenon, which can be solved only by a study of the pathophysiology of the process. The reduction in the incidence and severity of both the small- and large-vessel pathology in diabetes may call for a considerable modification of today's diabetic therapy.

The life expectancy of diabetics is increasing, and so is the problem of their employment. These sociological considerations have become the physician's problem as well as the patient's and are also discussed.

We then turn our attention in these pages to the more basic scientific advances and the understanding of the action of insulin and compare it with that of the sulfonylureas and biguanides. Having dealt with these fundamental aspects we finally launch a survey of the present status of the clinical use of hypoglycemic agents other than insulin in the management of the diabetic.

I can find no more suitable introduction to this publication than the statement made by Claude Bernard in 1877:[2] "If in the field of diabetes all the pathological obscurities have not as yet been completely elucidated, this is because the knowledge of the normal function is still imperfectly understood. Only by increasing our knowledge of the physiological aspects shall we find the basis for the formulation of an adequate pathological theory and by this dual approach we shall eventually master the control of the morbid phenomena. Physiology defined in this framework will be the only certain guide to the understanding of the pathology and the therapy of diabetes mellitus." It is in the tradition of this great man that the contributors to this monograph, representing all levels of investigation, basic and applied, furnish an impetus to the advancing knowledge of diabetes and its complications.

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Part I. Diabetes Mellitus: Certain Fundamental Clinical and Socioeconomic Considerations

THE GENETICS OF DIABETES: A REVIEW*

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According to Foster (1912), the earliest report of a familial incidence of diabetes mellitus was by Morton in 1696. Morton reported a family in which four children were diabetic. It seems reasonable to assume that the familial nature of diabetes was known long before this, because diabetes mellitus was apparently recognized by the sweet taste of the urine as early as 500 A.D. (Saundby, 1908). Be that as it may, daily experience early convinced clinicians that diabetes mellitus is frequently familial. It was not until the fourth decade of this century, however, that it was quantitatively established that the increased incidence is statistically significant (Pincus and White, 1933). These observations strongly indicated that susceptibility to diabetes is genetically determined because the necessary and sufficient criterion for establishing the presence of a genetic component in the causation of a character (disease) is an increased familial incidence in the absence of environmental factors that can explain this increase.

Further evidence for the hereditary nature of diabetes mellitus is offered by the many studies of twins (Then Berg, 1938; Joslin and White in Joslin *et al.*, 1946; and many others). In general, these studies show a greater frequency of concordance among monozygous twins than among dizygous ones. Since monozygous twins are genetically identical, and dizygous twins are no more alike than ordinary sibs, these observations indicate a genetic factor in determining susceptibility to diabetes. Concordance among identical twins is not complete; hence, postnatal environmental factors are important in determining the occurrence of frank diabetes.

Pincus and White (1933) showed that the familial incidence of diabetes may be explained by assuming that susceptibility to diabetes is due to homozygosity for a recessive gene. Independently, and at essentially the same time, William Allan (1933) arrived at the same conclusion from his highly skilled and too-frequently overlooked analysis of the family data from 143 cases.

These pioneering studies have been followed by numerous studies of large samples. The several investigators have advanced a variety of interpretations to explain their data. Prominent among them are hypotheses that distinguish the early-onset, severe, brittle diabetes from the late-onset, mild, stable type. These hypotheses are attractive on a priori grounds because of the clinical heterogeneity of the disease.

Three broad categories of diabetes appear to be recognized: (1) juvenile, with acute onset, severe course, and sensitivity to insulin; (2) late, with gradual onset, generally in obese individuals, mild course, and often no need of insulin;

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* The work reported in this paper was supported in part by Research Grants from the National Heart Institute and the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

and (3) J-type, with onset in young, thin individuals, insensitivity to insulin, and generally absence of ketotic reactions (Hugh-Jones, 1955).

The heterogeneity of the disease leads geneticists to expect that more than one genetic mechanism is concerned in the determination of susceptibility to diabetes. This suspicion is based on the experience that physiologically different characters are determined by different genes and, vice versa, that different genes causing similar appearances (phenotypes in genetic terms; syndromes, diseases, abnormalities in clinical terms) do so by different biochemical or physiological mechanisms.

Insofar as I am aware, no genetic studies have been made of the J type of diabetes, but many have been made of the other two varieties.

I have already mentioned the work of Pincus and White (1933, 1934) and of Allan (1933). Cammidge (1929, 1934) examined the pedigrees of 1000 diabetic patients. He concluded that early-onset diabetes is due to a recessive gene, and that late-onset diabetes is due to a dominant gene; presumably these are not alleles. Hanhart (1950) believes that all diabetic pedigrees may be explained by assuming recessive inheritance due to genes at one or more genetic loci, but neither Hanhart nor Cammidge has employed quantitative methods to analyze his material. Penrose and Watson (1945) reported the presence of a sex-linked tendency in susceptibility to diabetes, but Thompson and Watson (1952), in a subsequent study on a considerably larger sample that included at least a part of the sample studied by Penrose and Watson, failed to confirm the finding of a sex-linked tendency. It is noteworthy that all other investigators have also failed to confirm this finding. Thompson and Watson (1952) concluded that their data were ". . . suggestive of inheritance by a single autosomal recessive gene."

Ilse von Kries (1953), in a study of the families of 1305 probands, concluded, in agreement with Levit and Pessikova (1934), who studied the families of 222 probands, that diabetes is due to a dominant gene with incomplete penetrance. She based her conclusion, as did Levit and Pessikova (1934), on the observation that, in her sample and in that of some other workers, the frequency of diabetes among the parents of the probands was about equal to that among the sibs. Correction for the difference in age between parents and offspring would probably alter this situation. Since neither von Kries (1953), nor Levit and Pessikova (1934) examined the effect of parental diabetes on the frequency of diabetes among the offspring, their conclusions must be considered as being unfounded.

Grunnet (1957) published an extensive monograph based on his study of the families of 261 probands. He summarized his conclusions as follows:

"It is presumed that the majority of cases of diabetes mellitus are hereditarily conditioned and that a lesser percentage is acquired (exogenically conditioned).

"On account of the various mechanisms in the development of the disease, no single responsible mutating gene can be reckoned upon among the inherited cases, but several genes which individually or together can cause diabetes mellitus must be presumed.

"A part of the mild cases of diabetes in elderly individuals are probably exogenically conditioned.

"The majority of all cases of diabetes mellitus, in any case the majority of the severe cases, must be regarded as one group of primary pancreatogenic diabetes, probably inherited recessively."

The reasons for Grunnet's conclusions remain obscure to me. At no time are the data subjected to genetic analysis, not even to the simple extent of comparing the frequency of diabetes among the sibs of those with an affected parent with that among the sibs of those with healthy parents. Unfortunately, despite about 65 pages of tables and graphs, it is not possible to determine the number of sibs of each patient or the distribution of family size. The data therefore must remain unanalyzed.

Genetic Pattern

I have elsewhere analyzed in detail the studies published prior to 1952 (Steinberg and Wilder, 1952*b*). The objection to the conclusion that diabetes mellitus is due to a dominant gene rests upon the observation that, in all sets of data that are presented so that the comparison can be made, it is found that twice as many of the probands' sibs are diabetic if one parent is diabetic than if neither parent is diabetic. Dominant inheritance does not lead to such a pattern.

It is possible to elaborate a two-gene system that would approximate the data. Such a system would require a modifying gene that increases the penetrance of the dominant gene that leads to diabetes. Several unknowns that may be manipulated at will are inherent in such a hypothesis. Three of these are: (1) the frequencies of the alleles at each locus, (2) the penetrance of the supposed dominant gene in the heterozygous and homozygous states in the absence of the modifier, and (3) the modification of the penetrance of this gene, when heterozygous and when homozygous, by the modifier when heterozygous and when homozygous. It seems advisable to avoid such complicated explanations, especially when we have no way of testing them.

Despite these objections to dominant inheritance of diabetes as a generalization, the possibility remains that in rare instances diabetes is due to a dominant gene. Burnstein and Patterson (1949) published a remarkable pedigree extending over 5 generations and purporting to show dominant inheritance of a mild diabetes with clear expression of anticipation. N. Burnstein, Veterans Administration Hospital, Jackson, Miss., generously made it possible for me to correspond with the proband. The patient, a registered nurse, recompiled her family history for me by corresponding with various of her relatives. I am indebted to Burnstein and to the patient for their fine cooperation. Suffice it to say that there is a sufficient number of disagreements between the two pedigrees (the original and the recompiled) and a sufficient number of internal inconsistencies within the second pedigree to cast doubt on the validity of the data. The discrepancies concern the presence or absence of diabetes (54 diabetic in the published pedigree and 66 in the recompiled pedigree), age at onset, and even the existence of individuals. Among the inconsistencies were the ages of mothers at the birth of their children; for example, in one family, by using the reported age at death and date of death of the mother, and the ages of her children, we find she was 8, 18, 16, 18, and 10 years of age, respectively, at the birth of her first 5 children. A similar pattern occurred in other families. It is worth noting also that in the recompiled pedigree 54 of 66 reported diabetics are said to have become diabetic in an even year of life, and that 5 of the 12 with onset in an odd year of life became diabetic in a year of life ending in 5, and that in the original pedigree 46 of 54 reported diabetics were said to have become diabetic in an even year of life, and 4 of the 8 with onset in an odd year of life became diabetic in a year of life ending in 5.

The discrepancy in the number of diabetics in the 2 pedigrees has already been mentioned; we are concerned now with the peculiar distribution of the ages at onset. In an unbiased sample, equal numbers with onset in odd- and even-numbered years of life would be expected, and only one fifth of those with onset in an odd-numbered year of life would have onset in a year ending in 5. It is apparent that neither of the compilations of this pedigree is free of bias.

Mild diabetes may be due to a dominant gene in this family, and anticipation may have occurred in this family, but more detailed examination of the pedigree is required before the observations can be accepted.

Harris (1950), reasoning in the same manner as Wiener (1936) did for asthma, suggested that early-onset diabetes is due to the homozygous condition of a gene which, in the heterozygous state, leads to late-onset diabetes. Harris added that the distribution of the homozygous and heterozygous types would overlap to a certain extent with regard to age at onset and to severity. In addition there would be incomplete penetrance, particularly of the heterozygote.

TABLE 1
FREQUENCY OF DIABETES AMONG THE PARENTS OF PATIENTS WITH EARLY AND LATE ONSET OF DIABETES*

Patient's age at onset (years)	Per cent of diabetic parents	
	Harris	Steinberg and Wilder
0–29	3.3	5.0
30–	6.2	11.4

* Data from Steinberg and Wilder, 1952*b*.

Harris did not subject this attractive hypothesis to genetic or statistical tests. Unfortunately, such tests indicate that the hypothesis does not agree with the data. It may be shown that, on the basis of Harris's hypothesis, diabetes should be more frequent among the parents of diabetics with early onset than among the parents of patients with late onset. Steinberg and Wilder (1952*b*) showed that this was not the case in either Harris's data or their own (TABLE 1). It is disappointing that other sets of data have not been published in a manner that permits them to be similarly analyzed.

It may also be shown (Steinberg and Wilder, 1952*b*) that on Harris's hypothesis more of the sibs of the probands should be diabetic when onset in the proband was early than when it was late, and that this frequency should not be influenced by the presence or absence of diabetes in a parent. The data of TABLE 2, based on the samples of Harris (1950), of Thompson and Watson (1952), and of Steinberg and Wilder (1952*b*), show that these expectancies are not fulfilled. The frequency of diabetes among the sibs of the patients is essentially the same, regardless of the age of the proband at onset, and is about twice as frequent among those with an affected parent as among those with nondiabetic parents.

It appears from the foregoing that genetic susceptibility to diabetes is unlikely to involve sex-linked genes, or to be due to a dominant gene with incomplete penetrance, or to a gene that leads to late-onset, mild diabetes when heterozygous and to early-onset, severe diabetes when homozygous. The only simple genetic pattern left to consider is recessive inheritance.

It can be shown (Dahlberg and Hultkranz, 1927; Steinberg and Wilder, 1952*b*) that the relative frequencies of matings with none, one, and both partners affected among the parents of individuals homozygous for a recessive gene is as $p^2:2pq:q^2$, respectively, where q equals the frequency of the recessive gene (conveniently, the proportion of affected parents in the sample of parents equals q). These ratios are independent of age at onset, provided the distribution of age at onset is the same for the offspring of the three types of matings, and provided that only a relatively small proportion of the nondiabetic parents may be genetically liable to diabetes. These conditions appear to be fulfilled by those samples published in sufficient detail to permit such an analysis.

TABLE 2

FREQUENCY (IN PER CENT) OF DIABETES AMONG THE SIBS OF PATIENTS WITH EARLY AND LATE ONSET OF DIABETES

Patient's age at onset	All sibs		Neither parent diabetic Sibs		One parent diabetic Sibs	
	Total	Per cent diabetic	Total	Per cent diabetic	Total	Per cent diabetic
Harris (1950)						
0–29	1,019	4.1	971	3.5	48	18.8
30–	2,773	4.4	2,446	4.1	327	10.7
Thompson and Watson (1952)						
0–29	482	7.5	425	6.4	57	15.8
30–	4,125	9.1	3,411	7.8	714	15.3
Steinberg and Wilder (1952*b*)						
0–29	828	6.0	736	5.0	92	14.1
30–	7,456	6.0	5,928	4.6	1,528	11.2

TABLE 3 presents the comparison of the observed and expected numbers of the 3 types of mating for each of 6 sets of data. The χ^2 test of goodness of fit indicates that differences between the observed and expected numbers have a high probability of occurring by chance in 5 of the 6 samples. The sixth sample has been shown (Steinberg and Wilder, 1952*a*) to have certain inconsistencies with reference to ages at onset, which suggest that this set of data may not be as suitable for analysis as the remaining 5 sets. We may conclude, therefore, that the only simple genetic hypothesis consistent with the data is that susceptibility to diabetes is due to the homozygous condition of a recessive gene.

The way the data have been collected does not permit us to decide whether only one gene is involved or whether homozygosis for any one of two or more recessive genes may lead to diabetes. While it is a simple matter in experimental genetics to distinguish among these alternatives, it is difficult to do so in human genetics, particularly for a character with variable age at onset.

The method most likely to distinguish between the alternatives of a single locus versus two or more loci is to follow the offspring of families in which both parents are diabetic and that were selected via the parents. If only one locus is concerned, all children should be liable to diabetes and, if followed to a sufficiently advanced age, at least one child in each family should become diabetic. This would not be the case if two or more loci are involved. My colleagues, V. A. Newill, G. F. Badger, A. S. Littell, M. B. Sussman, and I are undertaking such a study as part of a broader study of diabetes. Others have attempted such studies in the past (Pincus and White, 1934; Steiner, 1936; Hanhart, 1950), but the data are not presented in sufficient detail to permit their evaluation.

TABLE 3

COMPARISON FOR 6 SETS OF DATA OF EXPECTED NUMBERS OF EACH OF 3 KINDS OF MATINGS YIELDING DIABETIC OFFSPRING WITH THE OBSERVED NUMBERS. THE EXPECTED NUMBERS ARE COMPUTED ON THE ASSUMPTION OF RECESSIVE HEREDITY

Mating	Steinberg and Wilder (1952*b*)		Pincus and White (1933)		Allan (1933)		Harris (1950)		Thompson and Watson (1952)		von Kries (1953)	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Neither parent diabetic	1589	1588.6	440	440.6	124	122.8	1124	1119.1	1404	1408.2	1137	1135.0
One parent diabetic	370	370.8	80	78.8	17	19.4	109	118.8	223	214.6	160	164.1
Both parents diabetic	22	21.6	3	3.6	2	0.8	8	3.1	4	8.2	8	5.9
Total	1981	1981.0	523	523.0	143	143.0	1241	1241.0	1631	1631.0	1305	1305.0
$\chi^2_{(1)}$	0.009		0.119		2.109		8.573		2.492		0.885	
p	>0.90		>0.70		>0.10		<0.01		>0.10		>0.30	

The ability to detect prediabetics would be a great asset in such a study. It is possible that the steroid-stressed glucose tolerance test (Berger, 1952; Fajans and Conn, 1954 and 1955; Conn, 1958) will be of help, and we plan to use it, but recent work indicates that negative results with this test may not be taken to indicate that the patient is not a prediabetic (West, 1957). Insofar as I am aware, Pincus and White (1934) are the only investigators to have used the glucose tolerance test to test a genetic hypothesis. These investigators tested the nondiabetic sibs of patients with none, one, or both parents diabetic. The frequencies of abnormal glucose tolerance tests among these sibs were 6.8, 17.9, and 25.0 per cent, respectively. These are in the ratio of 1:2.6:3.7, which makes them a close fit to the ratios expected on the assumption of simple recessive inheritance. The observed frequencies, however, fall short of the expected frequencies, which suggests that the test does not detect all presumed prediabetics. It would be of interest to know how the frequency of abnormal glucose tolerance tests varies with the age of those tested. Unfortunately, Pincus and White did not publish such data. A similar omission occurs in the data published by those using the cortisone-stressed glucose tolerance test (Conn, 1958; West, 1957).

If we accept the hypothesis that most if not all cases of diabetes are due to homozygosis for the same recessive gene, we can estimate the frequency of the gene in the population (Steinberg and Wilder, 1952*b*) and, using this estimate, the probability that various relatives of a diabetic patient would be liable to diabetes (Steinberg, 1955*a*, *b*). It is estimated that the gene frequency is about 22 per cent, and that about 5 per cent of our population is genetically liable to diabetes. Since only about 2 per cent of the population is diabetic (detected and undetected, Joslin *et al.*, 1946), it follows that the frequency of the disease may be expected to increase as our population ages. TABLE 4, from Steinberg (1955), presents a summary of the probability of being genetically liable to diabetes (*dd*) as a function of the relationship to a diabetic patient. The risk runs from less than 20 per cent to 80 per cent. The table does not include the risks incurred when a sib is diabetic because these are clearly 25, 50, and 100 per cent if neither, 1, or both parents, respectively, are diabetic.

TABLE 4

PROBABILITY THAT AN INDIVIDUAL WILL BE GENETICALLY LIABLE TO DIABETES IF HE HAS 1 OR MORE DIABETIC RELATIVES*

Probability that a person is genetically liable to diabetes†	Diabetic relatives
Up to and including 20 per cent	(1) First cousin, (2) uncle or aunt, (3) one grandparent, (4) two grandparents (spouses) or one affected parent.
30 to 40 per cent	(1) Two grandparents (not spouses) (2) one parent and first cousin on nondiabetic parent's side.
50 to 80 per cent	(1) One parent and a sib of the nondiabetic parent, (2) one parent and a parent of the nondiabetic parent, (3) one parent and a sib and a parent of the nondiabetic parent.

* Relatives refers to parents, grandparents, aunts, uncles, and first cousins.

† Within each group the relatives are listed so as to yield an increasing probability that the person is liable to diabetes (*dd*). Thus, an individual with only a first cousin diabetic (1 of the first group) has the lowest probability of being *dd* while a person with one parent, (3 of the last group) has the highest probability of being *dd*.

While it is of some use to be able to offer probabilities of being liable to diabetes, it would be much more satisfactory to be able to predict ages at onset and to be able to say that after a given age the risk is essentially nil. The possibility of doing this was raised by Woodyatt and Spetz (1942), who concluded from their study of 100 pairs of parent and child diabetics that the average age at onset decreases 20 years per generation, and that a child would rarely if ever become diabetic at a greater age than had his parent. This is known as anticipation. Steinberg and Wilder (1950 and 1952*a*) showed that, among children of diabetics, the younger age at onset of diabetes is a statistical and not a biological phenomenon. These conclusions were based on an analysis of 200 parent-child pairs for which the ages at onset were known for both parent and child.

In their paper on the genetics of diabetes, Steinberg and Wilder (1952*b*) presented data for 301 parent-child pairs; these include the 200 pairs previously reported (1952*a*). Table 5 presents a summary of these 301 pairs. Onset was in a prior decade of age in 63.4 per cent of the offspring. Hence, as Woodyatt and Spetz reported, prior onset occurs in the majority of the diabetic children of diabetic parents. It may be demonstrated, however, that this frequency of prior onset is precisely what would be expected to occur by chance if we assume that there is no biological relation between the age at onset in parent and child (Steinberg and Wilder, 1950 and 1952*a*).

Table 5

Number of Patients Whose Age at Onset Was in a Decade Prior to that at Onset of the Diabetic Parent*

Age of parent at onset	Total No. of parent-child pairs	No. of patients with onset in a prior decade of life
20 to 29	3	1
30 to 39	15	5
40 to 49	51	18
50 to 59	93	43
60 to 69	85	71
70 to 79	41	40
80 to 89	13	13
Total..................	301	191

Per cent prior onset = (191/301) × 100 = 63.4

* Data from Steinberg and Wilder 1952*b*.

Table 6

Derivation of the Expected Frequency of Prior Onset Among 301 Patients (Values Are Percentages)

Age in years	Cumulative distribution of age at onset*	Distribution of age at onset among 301 parents	Expected frequency of prior onset in the children
0 to 9	4.9	—	
10 to 19	11.8	—	
20 to 29	19.4	1.0	0.1
30 to 39	31.6	5.0	1.0
40 to 49	54.1	16.9	5.3
50 to 59	81.1	30.9	16.7
60 to 69	96.4	28.2	22.9
70 to 79	98.8	13.6	13.1
80 to 89	100.0	4.3	4.2
Total..............		99.9	63.3

* Based on data of 12,740 patients published by Joslin *et al.* (1946).

The calculations are illustrated in Table 6. The expected frequency of children with prior onset for each decade of onset in the parent is obtained by multiplying the value for the proportion of parents with onset in a given decade by the percentage of all diabetics with onset before that decade. Thus, for parents with onset during the fifth decade (40 to 49 years), the expected percentage of children with prior onset is 0.169 × 31.6 = 5.3 per cent. If this operation is repeated for sets of parents with onset in each of the decades, the total expected frequency may be derived. This frequency, 63.3, is almost identical with the observed frequency, 63.4.

TABLE 7

AGE AT ONSET AND FREQUENCY OF PRIOR ONSET VERSUS DIABETIC PARENT

Diabetic patients	Diabetic parent	
	Mother (186)	Father (115)
Percentage with prior onset	63.4	63.5
Mean age at onset in years (a)*	49	48
Mean age at onset in years (b)†	42	39

* (a) All patients with a diabetic parent.
† (b) Patients whose diabetic parents became ill before age 50: 44 mothers, 23 fathers.

TABLE 8

FREQUENCY OF DIABETES AMONG THE SIBS OF DIABETIC PATIENTS AS A FUNCTION OF THE SEX OF THE DIABETIC PARENT*

Diabetic parent	Number of patients	Sibs		
		Total	Diabetic	
			No.	Per cent
Mother	220	1000	113	11.3
Father	150	620	72	11.6
Total	370	1620	185	11.4

* Data from Steinberg and Wilder (1952*b*).

It has been shown that neither the diabetic nor the prediabetic state in the mother influences the age at onset or the probability of occurrence of diabetes in the child (Steinberg and Wilder, 1952*a*; Steinberg, 1955*b*). TABLE 7 presents a summary of the data, evaluating the possibility of a maternal effect on the age at onset in the child. TABLE 8 presents the data showing the frequency of diabetes among the sibs of the probands as a function of the sex of the diabetic parent.

In this sample the mean difference between the ages at onset of the parents and the probands is 19 years. It may be shown by calculations similar to, but more detailed than, those illustrated in TABLE 6 that the expected advance in age (assuming independence of age at onset of parent and child) is also 19 years (Steinberg and Wilder, 1952*a*).

We may conclude, therefore, that anticipation has no biological significance, and that we cannot use the parent's age at onset to predict the child's age at onset or the end of the period of risk for the child.

If we assume that the distribution of the age at onset is essentially the same for all segments of the population of the United States, we may use the cumulative distribution of the age at onset shown in column 1 of TABLE 5 to estimate the proportion of the period of risk that has been passed. For example, 54 per cent of those who will become diabetic are already diabetic by the age of 50 years; therefore, we may assume that 54 per cent of the risk has been run by this age. For the present, this appears to be about as well as the geneticist can do. More precise information for the patient will become available only when the prediabetic can be detected with a high degree of certainty.

Summary

It is demonstrated that susceptibility to diabetes is probably inherited as a recessive character and that this hypothesis is the only one consistent with the several large samples of family data that have been published.

It is not clear from the available data whether homozygosis at only one locus or at any one of two or more loci will cause susceptibility to diabetes.

It is demonstrated that anticipation is a statistical and not a biological phenomenon.

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THE EARLY RECOGNITION OF DIABETES MELLITUS*

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Progressively earlier recognition of the diabetic state is vital if progress is to be made toward the eventual control and prevention of the disease. The presence of mild diabetes mellitus may remain unrecognized in a large number of individuals for many years unless diagnostic laboratory procedures are freely employed in groups of individuals in whom experience has shown a high incidence of latent diabetes. Dependable diagnostic criteria are now available that make it possible to detect these previously unsuspected diabetic individuals. In addition, it is hoped that other means for detection of the diabetic state will be discovered that will give earlier evidence of its existence than is possible by present methods of testing.

This discussion has two purposes, first, to consider the use and the criteria for the interpretation of the standard oral glucose tolerance test employed for the early detection of diabetes and second, to consider experience with the cortisone-glucose tolerance test as used for the possible prediction of future diabetes mellitus.

The early recognition of diabetes mellitus depends upon the use and interpretation of proper laboratory procedures. In some cases the presence of latent diabetes will be recognized by routine performance of urinalysis and the determination of a single blood sugar level. In the mildest forms of the disease these procedures may be of little aid. In these cases the earliest recognizable abnormality may be a diminished ability to utilize a carbohydrate load as demonstrated by a glucose tolerance test.

We have used the oral glucose tolerance test in our studies. For at least 3 days preceding the test the subjects ingest a diet containing approximately 300 gm. carbohydrate per day plus maintenance calories.[1] Although the ingestion of smaller amounts of carbohydrate is sufficient to prevent abnormal tests in normal subjects, a standardized high-carbohydrate preparatory diet is advised to ensure the reproducibility of the test. The loading dose of glucose used is 1.75 gm./kg. of ideal body weight. Blood sugar levels are determined in venous blood by the Somogyi-Nelson technique.

The criteria that we employ for the interpretation of the standard oral glucose tolerance test are illustrated in FIGURE 1.[2] Carbohydrate tolerance is considered to be normal when the peak blood sugar value is less than 160 mg./100 ml. and the 2-hour value is less than 110 mg./100 ml. We regard the combination of an 1-hour value of 160 mg./100 ml. or above plus a 2-hour value of 120 mg./100 ml. or above as diagnostic of the existence of the diabetic state. In borderline curves the level at 1½ hours is required to be 140 mg./100 ml. or above to be diagnostic. This restriction eliminates a false diagnosis of

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* The work reported in this paper was supported in part by Research Grant A-888 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

diabetes on the occasional curve that drops abruptly to normal at 1½ hours and then rebounds above 120 mg./100 ml. by 2 hours. Figure 1 shows that a diabetic curve is one in which all points lie at or above the upper border of the shaded triangle. A diagnosis of "probable diabetes" is made when the 1-hour value is 160 mg./100 ml. or above, the 1½-hour value 135 mg./100 ml. or above, and the 2-hour value is between 110 and 120 mg./100 ml., which falls within the shaded zone of figure 1. It should be emphasized that these criteria for the interpretation of the glucose tolerance test are applicable only in ambulatory and otherwise healthy individuals.

The criteria described are similar to those of Moyer and Womack[3] and those of Mosenthal and Barry.[4] However, our criteria are less restrictive than those of Mosenthal and Barry, who considered carbohydrate tolerance to be abnormal when the maximum blood sugar value is above 150 mg./100 ml. and the 2-hour value above 100 mg./100 ml. Also, these workers did not employ a value at 1½ hours in mildly abnormal glucose tolerance tests to eliminate false positive rebound curves.

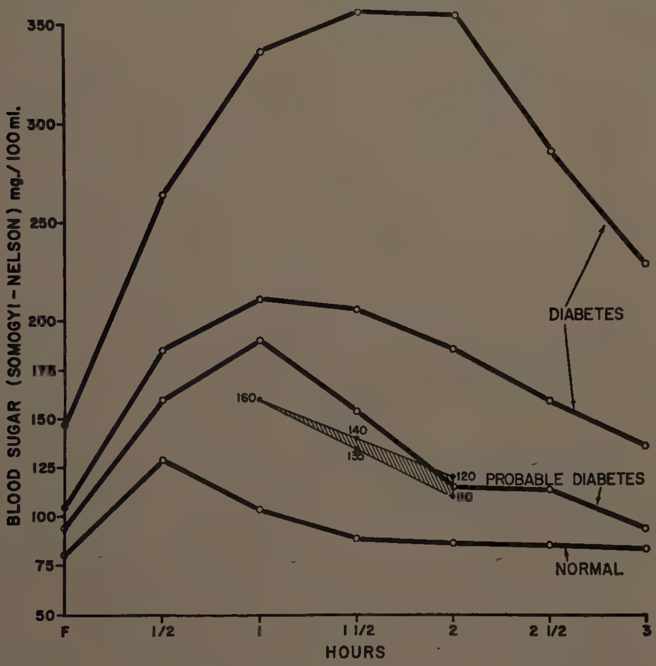

Figure 1. Criteria used for the interpretation of the standard oral glucose tolerance test.

It is obvious that any criteria which employ a sharp dividing line between normal and abnormal must be arbitrary. What, then, are the justifications that make us believe that our criteria are sound? Applying these criteria in 127 healthy subjects without a history of familial diabetes or large babies, we have found only one glucose tolerance test indicating diabetes and one indicating "probable diabetes." In addition, careful follow-up of patients with mild abnormalities of glucose tolerance frequently shows further impairment of carbohydrate utilization clearly diagnostic of diabetes mellitus. Decompensation of carbohydrate tolerance may occur rapidly, although in middle-aged individuals loss of glucose tolerance may be only slowly progressive over many years. The following examples will serve to illustrate the significance of mild abnormalities of glucose tolerance as defined by our criteria.

Subject A. N. (TABLE 1) shows a 1-hour blood sugar value of 177 mg./100 ml. and a 2-hour value of 121 mg./100 ml. A few days later, fasting hyperglycemia was diagnostic of diabetes mellitus. Patient K. C. (TABLE 2) is an example of "probable diabetes," since the 2-hour value is between 100 and 120/ mg./100 ml. Nine years later no doubt remains about the diagnosis of diabetes mellitus. In subject A. C. (TABLE 3) the diagnosis of probable diabetes could have been made in 1934. Nineteen years later he developed symptoms of frank diabetes, and diagnostic fasting hyperglycemia was found.

TABLE 1

PATIENT A. N., MALE, AGED 69 YEARS, HEIGHT 5′8″, WEIGHT 160 LB.

Date	Glucose tolerance test (hours)					
	Fasting	½	1	1½	2	3
9–14–58	94	171	177	188	121	143
9–17–58 (surgery)	124					
9–18–58	171					

TABLE 2

PATIENT K. C., MALE, HEIGHT 6′1″, FAMILY HISTORY OF DIABETES (MOTHER)

Year	Age	Weight	Glucose tolerance test (hours)									
			Fasting	½	1	1½	2	2½	3	3½	4	4½
1948	36	160	76		197		118	96	54	36	58	68
1957	45	183	119									
			115									
			100	208	286	260	216	(Diet discontinued by patient after 3 months)				
1958	46	191	96	177	216		288		179			

The last 2 patients exhibited another manifestation of early diabetes mellitus. Terminal hypoglycemia 3 to 5 hours after ingestion of glucose occurs in a large number of individuals whose glucose tolerance tests disclose mild diabetes mellitus.[5] Occurrence of terminal hypoglycemia does not rule out the presence of diabetes mellitus. Symptoms of spontaneous hypoglycemia 3 to 5 hours after meals occur in many cases with early mild diabetes mellitus, and may indeed be one of the earliest clinical manifestations of the disease. Of a series of 110 patients with glucose tolerance tests that exhibited early hyperglycemia and late hypoglycemia, 44 per cent had a family history of diabetes mellitus.[6]

Several types of glucose tolerance curves that cannot be classified as indicating diabetes or probably diabetes according to our criteria, but which nevertheless may have prognostic significance, deserve comment. The diagnosis of diabetes mellitus cannot be made with confidence on the basis of an abnormal elevation of the blood sugar level at ½ or 1 hour if it is accompanied by a normal 2-hour blood sugar level during the glucose tolerance test. Rapid transit of glucose through the pylorus may give rise to abnormally rapid intestinal absorption of glucose and to high peak blood sugar values. However, careful follow-up of otherwise healthy individuals with abnormally high peak blood sugar levels during the glucose tolerance test is indicated. Table 4 shows the progression of such curves to curves diagnostic of diabetes mellitus.

Table 3
Patient A. C., Male, Height 6′0″, Glycosuria Found Twice in 1930

Year	Age	Weight	Glucose tolerance test (hours)			
			Fasting	1	2	3
1934	48	170	0 86	+ 205	+++ 115	++ 49
1953	67	182	Polyuria, polydipsea, 7-pound weight loss during preceding month			
11–17–53			++++ 220			
12–10–53			+++ 232			

Table 4
Prognostic Importance of Abnormally High Peak Blood Sugar Levels with Normal 2-Hour Levels

Subject and height	Date	Age	Weight	Glucose tolerance test (hours)						
				Fasting	½	1	1½	2	2½	3
M. P., male, 6′	1935	43	160	0 130		++++ 174		++++ 93		0 68
	1940	48	161	++++ 211						
A. M., female, 5′5″	3–5–53	33	160	0 100		++ 180		+ 96		+ 118
	6–25–53	33	162	123	214	259	189	158	163	137
	3–23–59	39	148	136	240	280	264	180	193	137
J. G., male, 5′10″	3–15–55	24	178	0 98	+++ 198	+++ 153		+++ 76		0 56
	6–1–55	24	177	0 88	188	+++ 207	+++ 214	+++ 218	143	+++ 140

Renal, or nondiabetic, glycosuria has been thought to be a benign condition. A majority of authors have reported the development of diabetes in such individuals to be no more frequent than in the general population. However, we have found unsuspected diabetes in 63 per cent of the patients with nondiabetic glycosuria in whom follow-up glucose tolerance tests were performed up to 30 years later.[6] Approximately one third of the group had initially exhibited fasting glycosuria. This fact indicates that nondiabetic, or renal, glycosuria may be indicative of potential diabetes, and that such patients should be retested periodically for the presence of diabetes mellitus. TABLE 5 shows examples of the transition from nondiabetic glycosuria to diabetes mellitus.

Mild variations of glucose tolerance may be encountered when the test is repeated at intervals of more than a few days in the same individual. In general, however, there is a high degree of reproducibility when the oral glucose tolerance test is carefully performed in conjunction with the use of a standard high-carbohydrate preparatory diet and a true blood sugar method. Occasionally one encounters a rapid change from an abnormal glucose tolerance test to a normal one. It has been recognized for many years that reduction of body weight in the obese middle-aged diabetic patient may result in a return to normal of the glucose tolerance test.[7] It is less well recognized that spontaneous reversal of an abnormal glucose tolerance test to normal may also occur in young untreated diabetics; thus, a change from a glucose tolerance test diagnostic of diabetes, by the criteria given, to a normal one does not invalidate these criteria per se. An extreme example of such a change to a nondiagnostic glucose tolerance test with subsequent development of severe diabetes with complications is shown in TABLE 6.

TABLE 5
TRANSITION FROM NONDIABETIC GLYCOSURIA TO DIABETES MELLITUS

Subject and height	Year	Age	Weight	Glucose tolerance test (hours)						
				Fasting	½	1	1½	2	2½	3
A.B., female, 5′4″	1936	22	114	0 89		+++ 135		++ 104		0 64
	1948	34	115	++++ 260	(Polyuria, polydipsea, weight loss)					
F. J., male, 6′0″	1937	48	190	++ 97		++++ 107		++ 91		++ 68
	1956	67	190	++ 97	+++ 188	+++ 186	++++ 151	+++ 136	++++ 127	+++ 102

Having discussed the criteria for the interpretation of the oral glucose tolerance test and the significance of mild abnormalities of carbohydrate tolerance, we indicate one use of this test as it applies to the early detection of diabetes mellitus. FIGURE 2 shows the results of the standard glucose tolerance test when performed in 438 close relatives (parents, siblings, or children) of known diabetic patients. Of this group, 340 (77 per cent) had normal glucose tolerance curves. Curve 1 is the composite curve of this group. Of the 438, 81 (19 per cent) were found to be diabetic. Curve 2 is the composite curve for these previously unsuspected diabetics. Seventeen of the 438 relatives, (4 per cent) gave curves indicating probable diabetes (curve 3). In contrast, using the same criteria in a control group of 127 subjects without a family history of diabetes or of large babies, only one previously unrecognized diabetic (Curve 2) and one probable diabetic (Curve 3) were discovered. The other 125 subjects, (more than 98 per cent) had normal glucose tolerance tests. Thus, at least a 19 per cent incidence of previously unsuspected diabetes can be found in close relatives of diabetic patients, while this incidence is less than 1 per cent in the control group. A very much higher incidence of previously unrecognized diabetes will be found if studies are conducted among close relatives of diabetic patients than in the general population.

TABLE 6

REVERSIBILITY OF DIAGNOSTIC GLUCOSE TOLERANCE TEST

B. S., male: height 5′6″, weight 152 lb.
3–16–38 Glucosuria found, but no symptoms

Date	Age	Glucose tolerance test (hours)			
		Fasting	1	2	3
3–18–38	24	0 214	375	353	273
3–29–38	24	0 107	146	112	99
1946	32	Furunculosis, polydipsea, polyphagia, 8-lb. weight loss; recovered without treatment.			
1947	33	Abdominal wall abcess, fatigue: *FBS*—350 mg./100 ml. Diagnosis: Diabetes mellitus. Diet and insulin.			
1948	34	Retinitis proliferans, vitreous hemorrhages.			

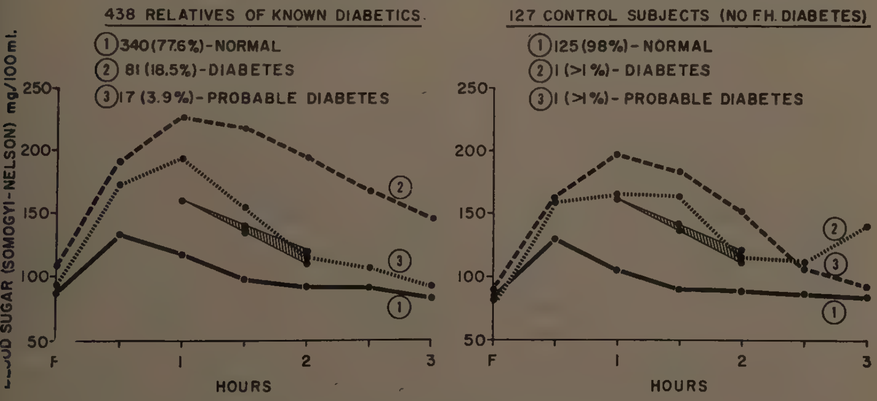

FIGURE 2. Standard oral glucose tolerance tests.

To develop means of recognizing the potential diabetic is the aim of our current investigations. The cortisone-glucose tolerance test has been used as a test for determining the potentiality for the development of the disease.

When this test is applied to nondiabetic relatives of diabetic patients it may serve as a means for predicting future diabetes.[2, 8, 9]

We used the following technique for the performance of the cortisone-glucose tolerance test in our studies. An individual weighing less than 160 lb. receives orally 50 mg. of cortisone acetate 8½ hours and again 2 hours before the ingestion of glucose. If body weight exceeds 160 lb., 62½ mg. cortisone acetate is given orally at the same time intervals. A value of 140 mg. per cent at 2 hours is the critical level for interpretation of the cortisone-glucose tolerance test. Thus, a cortisone-glucose tolerance test giving a 2-hour blood sugar level of 140 mg. per cent or above (and therefore lying at or above the line connecting the points of 160 mg. per cent at 1 hour and 140 mg. per cent at 2 hours in FIGURE 3), represents a "positive response," while a curve with a level of below 140 mg. per cent at 2 hours is regarded as a "negative response."

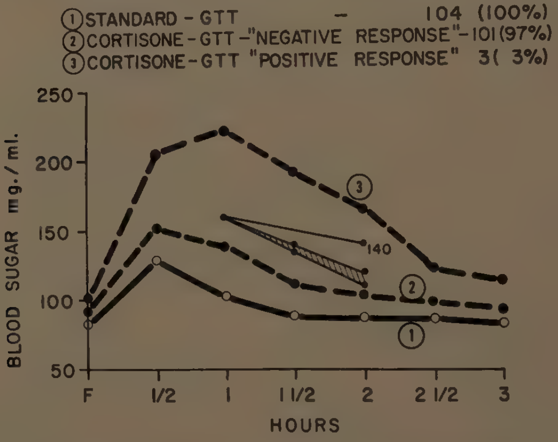

FIGURE 3. Cortisone-glucose tolerance tests on 104 healthy subjects with no family history of diabetes or large babies.

FIGURE 3 illustrates our findings with the cortisone-glucose tolerance test in a group of 104 control subjects without a family history of diabetes or large babies. Curve 1 demonstrates their composite standard glucose tolerance test before cortisone was given. After administration of cortisone 101 subjects or 97 per cent of the control group gave "negative responses" to this test with a mean 2-hour blood sugar of 104 mg./100 ml. (Curve 2). Three subjects (3 per cent) of the group gave a "positive response" to this test.

Cortisone-glucose tolerance tests were performed in 295 nondiabetic relatives of known diabetics. All of these subjects had normal control glucose tolerance tests as shown in FIGURE 4. After administration of cortisone 220 of the 295 individuals (75 per cent) showed a negative response with a mean 2-hour blood sugar level of 106 mg. per cent, a response seen in 97 per cent of the control group. Seventy-five of the 295 subjects (25 per cent) showed a positive response. Thus, 25 per cent of the group of nondiabetic relatives respond to this test as do only 3 per cent of the control group.

FIGURE 5 shows the data obtained with 17 subjects classified by their initial glucose tolerance test as individuals with probable diabetes (Curve 1). Fifteen of the 17 (88 per cent) gave positive responses to the cortisone-glucose tolerance test, while 2 of the 17 gave negative responses to it.

Figure 6 demonstrates data obtained in 22 obese, mild diabetic patients. Curve 1 shows the composite glucose tolerance test before reduction of body weight. After weight reduction all subjects exhibited normal standard glucose tolerance curves, as shown in Curve 2. Cortisone-glucose tolerance tests were then performed. Of the 22, 19 (86 per cent) gave a positive response to the test (Curve 3).

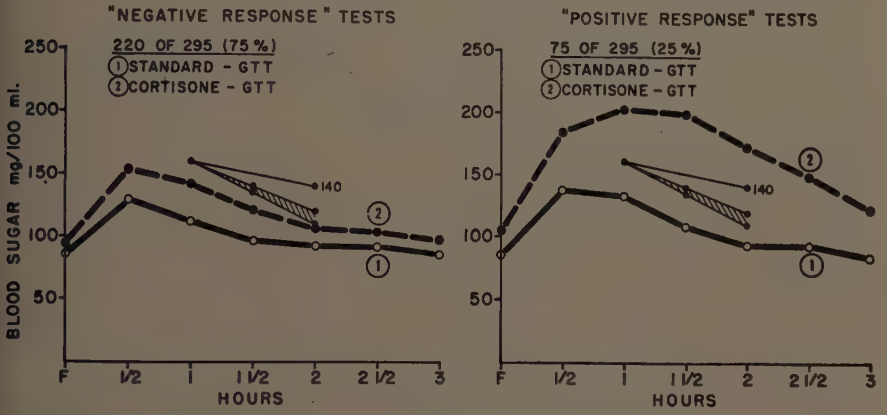

Figure 4. Cortisone-glucose tolerance tests on 295 nondiabetic relatives of diabetic patients.

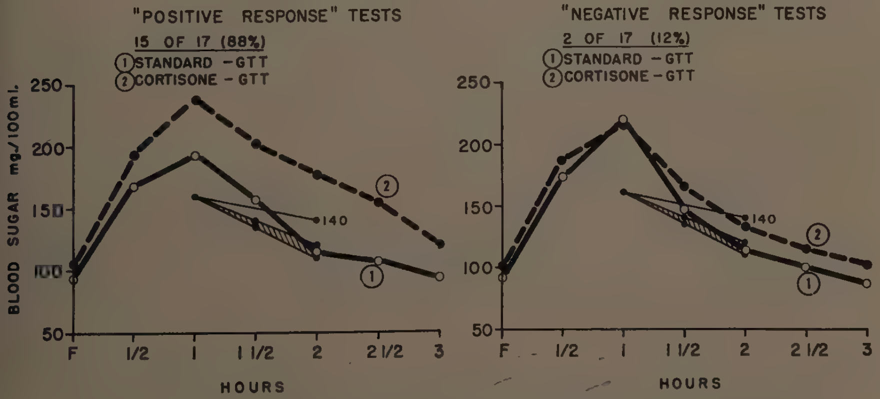

Figure 5. Cortisone-glucose tolerance tests on 17 subjects with probable diabetes.

In correlating these results it seems very significant that 25 per cent of the group of nondiabetic relatives of diabetic patients respond to this test in the same way as do 88 per cent of patients with probable diabetes and 86 per cent of the group of obese diabetics whose carbohydrate tolerance had returned to normal after reduction of body weight. On the other hand, only 3 per cent of the control group without a family history of diabetes gave a positive response to the cortisone-glucose tolerance test.

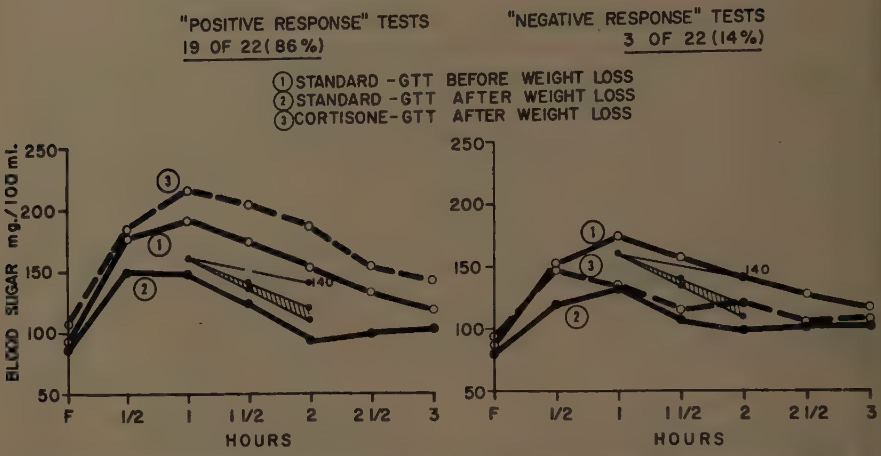

FIGURE 6. Cortisone-glucose tolerance tests on 22 obese diabetic subjects after loss of body weight.

TABLE 7
FOLLOW-UP (1 TO 6 YEARS) RESULTS IN 91 NONDIABETIC RELATIVES OF DIABETIC PATIENTS

Initial normal GTT		Progression to:		
Cortisone-GTT	Number subjects	Diabetes number	Probable diabetes number	Total abnormal number
Positive response	34	6 (18%)	4 (12%)	10 (30%)
Negative response	57	1 (2%)	0	1 (2%)

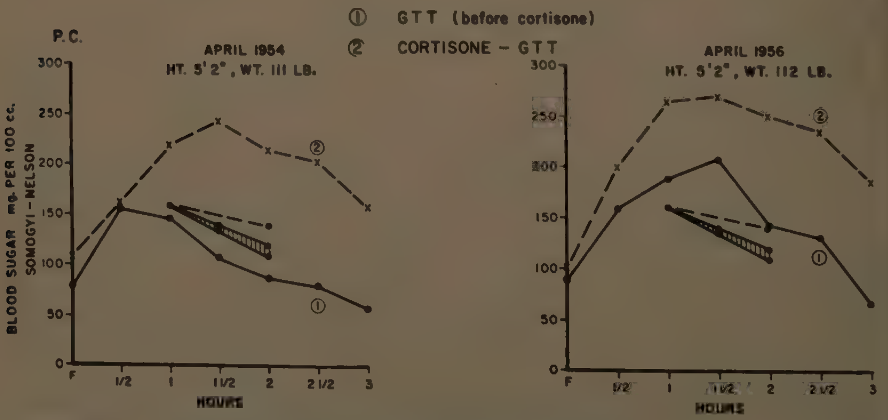

FIGURE 7. Glucose tolerance tests and cortisone-glucose tolerance tests in a 32-year-old female patient with familial diabetes mellitus (father and brother).

Thus, using this test the presently nondiabetic relatives of diabetic patients can be divided into two groups: "Positive response" and "negative response." Time and careful follow-up will determine the incidence of future diabetes in each of the two groups. Such studies have been in progress in 91 nondiabetic relatives of diabetic patients over the last 6 years (TABLE 7).

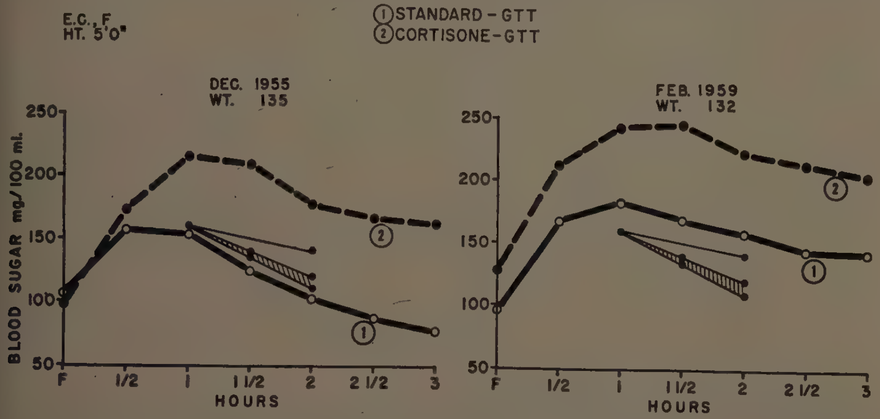

FIGURE 8. Glucose tolerance tests and cortisone-glucose tolerance tests in a subject with amilial diabetes mellitus (mother).

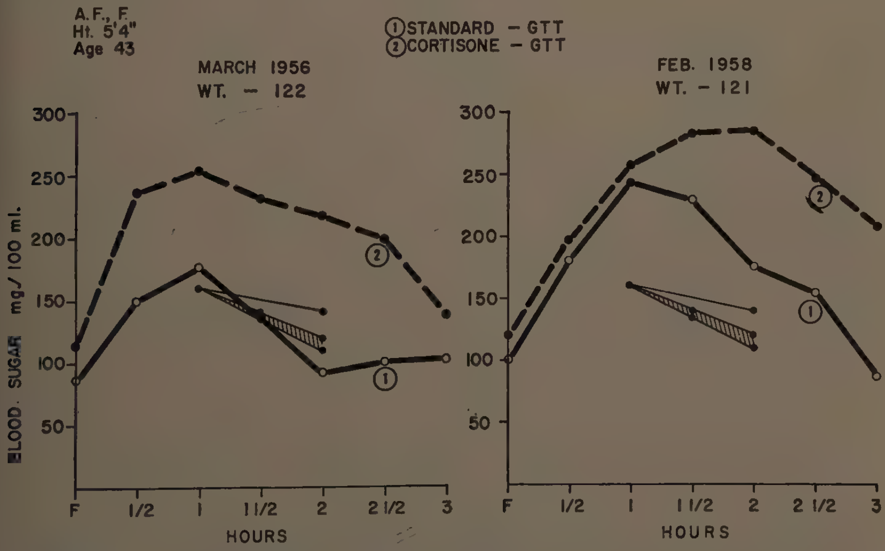

FIGURE 9. Glucose tolerance tests and cortisone-glucose tolerance tests in a subject with familial diabetes mellitus (mother, 5 siblings).

Of the 91 subjects, 34 had positive responses to the cortisone-glucose tolerance test at the time of the initial tests. Subsequently, 6 (18 per cent) of these 34 subjects have developed diabetes, and another 4 (12 per cent) have probable diabetes. Of the 57 subjects with negative responses to the cortisone-glucose tolerance test, only 1 has developed diabetes. In summary, 30 per cent of the positive-response group has developed diabetes or probable diabetes, while only 2 per cent in the negative-response group yielded an abnormal glucose tolerance test at a later date. Thus, the cortisone-glucose tolerance test may prove to be of value as a test for the prediction of future diabetes. FIGURES 7 and 8 give examples of the progression from a normal glucose tolerance, but abnormal cortisone-glucose tolerance, test to diabetes mellitus in the course of 2 to 4 years. FIGURE 9 illustrates an additional example. Although the initial standard glucose tolerance test could not be classified as one demonstrating diabetes or probable diabetes, it exhibited a mild elevation of the blood sugar level at 1 hour. The cortisone-glucose tolerance test was strongly positive. Two years later the patient was diabetic. This result reemphasizes the possible prognostic importance of an elevated peak blood sugar level.

Summary

The diagnostic criteria that we employ for the interpretation of the standard oral glucose tolerance test have been reviewed.

Unsuspected diabetes has been found in 19 per cent of 438 close relatives of diabetic patients, while another 4 per cent fell into a category that we have termed "probable diabetes." When these criteria are applied to 127 healthy subjects without a history of familial diabetes or large babies, only 1 glucose tolerance test indicating diabetes and 1 indicating probable diabetes have been found.

Positive responses to the cortisone-glucose tolerance tests have been obtained in 3 per cent of the control group without a family history of diabetes, in 25 per cent of the nondiabetic relatives of diabetic patients, in 88 per cent of patients with probable diabetes, and in 86 per cent of a group of obese diabetic patients whose carbohydrate tolerance had returned to normal after reduction of body weight.

A positive response to the cortisone-glucose tolerance test in nondiabetic individuals can be interpreted as indicating a potentiality or susceptibility for the development of diabetes. Follow-up in 91 nondiabetic relatives of diabetic patients over 1 to 6 years supports this view.

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PREGNANCY AND THE PREDIABETIC STATE

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Quite appropriately it has been said that the ideal clinical state for the investigation of "prediabetes" is to be found in the pregnant woman.[1] Most of our knowledge and postulation have come from studies of her "career" and her offspring.

The investigations of Jackson,[2] Conn,[3] and Hoet[4] refer respectively to intriguing premises about the origin at birth, the earliest possible recognition of premonitory signs, and the possibilities of primary prevention of diabetes mellitus and its associated complications in pregnancy.

A basic premise by which an investigation might be guided may be that those who ultimately become overtly diabetic are born with a susceptibility or predisposition to the disorder, transmitted through heredity and/or by an abnormal metabolic intra-uterine environment. With the passage of time a susceptible human host ages, being subjected to cumulative environmental organic stresses and insults, including such things as obesity and infection and, if a woman, possibly subjected to the stress of repeated pregnancies. The time at which an irreversible state of diabetes mellitus will manifest itself may depend upon the extent of the basic pattern of susceptibility and the amount of pathology produced by each added stress or physiological insult.

Our present interest is concerned with pregnancy and the prediabetic state. In this regard several studies[5-8] made in retrospect and reported in medical literature conclude that the diagnosis of overt diabetes in many women is not made until months or even many years after these women have had certain characteristic experiences in pregnancy. These experiences, allegedly delineating the "prediabetic state," include a high incidence of big babies, stillbirths, neonatal deaths, spontaneous abortions, premature deliveries, toxemia, and congenital anomalies.

As a part of our premise, it is also felt that, during pregnancy, transient abnormalities of carbohydrate metabolism as detected by a standard oral glucose tolerance test may indicate that a diabetogenic effect is being exerted, not only on the mother, but also on the fetus.

Some of the abnormal experiences in pregnancies related subsequently to the prediabetic state are more pronounced than others. While the expected incidence of big babies (10 pounds or more as classically described in the literature) is only about 2 per cent in the general population,[9] these babies are reported in medical literature as being born to 20 per cent or more of prediabetic mothers. This experience is most often seen 10 to 20 years before diagnosis of overt diabetes in the mother.[6]

Fetal and neonatal mortality is reported in the high range of 20 to 40 per cent in the 5-year period immediately preceding the development of overt diabetes, but it is increased appreciably over normal as much as 30 years prior to the recognition of this metabolic disorder.[6]

White[10] reports toxemia in 10 per cent of cases, abortions in 42 per cent and perinatal loss in 30 per cent in the 10-year period immediately prior to as well as following the recognition of diabetes.

An increased incidence of congenital defects has been noted by some authorities, but not by others.[6]

The possibility that experiences such as those mentioned above could be harbingers of the ultimate disease is worthy of investigation. If the "prediabetic state" in pregnancy can be recognized by any one or combination of the phenomena alluded to, then early intervention to prevent or halt its progression can be attempted. One preventive measure considered for study is that the correction of abnormal metabolism during pregnancy by insulin administration may reduce some of the complications of pregnancy and/or delay the onset of diabetes.[4, 8]

Objectives and Plan of Study

Since 1954, in cooperation with the Massachusetts Department of Health, Boston, Mass., the Public Health Service of the Department of Health, Education, and Welfare, Washington, D. C.,[11] has conducted at the Boston City Hospital and the Boston Lying-In Hospital, Boston, Mass., a study of impaired CHO metabolism in pregnancy and the effect of the use of insulin in cases of such impairment. The Children's Bureau, Washington, D. C., has assisted by making available improved service facilities for the mothers and children in the project.

Three specific objectives are being sought. The first is to determine, if possible, the facts about glucose tolerance tests for abnormal CHO metabolism or "prediabetes" in pregnancy; that is:

(1) If women having such abnormal tests actually have a higher rate of oversize babies, fetal wastage, and complications of pregnancy than those with normal tolerance.

(2) If such women actually do develop frank diabetes in subsequent years at a higher rate than women with normal tolerance.

(3) If children born of mothers who have abnormal CHO metabolism in pregnancy actually do develop diabetes more frequently or have more abnormalities in their tolerance tests than children born of mothers with normal metabolism.

Second, an attempt is being made to determine if insulin treatment of such abnormal carbohydrate metabolism will alter or correct any of these abnormal outcomes of pregnancy mentioned above, if indeed they actually occur.

Third, it is hoped that a generally practical method of detecting such a "prediabetic state," if it actually exists, will evolve from the study as it is being performed.

At the time pregnant women register for prenatal care they are given a lemon-flavored drink of 50 gm. glucose. Immediately following this a history is taken of past pregnancy experiences and the occurrence of diabetes in the family. One hour after the glucose drink a venous specimen of blood is taken and analyzed for true glucose by the Somogyi-Nelson method. In this preliminary or first-stage study of pregnancy history and blood sugar, those women "screen positive" and are termed "positive screenees" who are identified as having any one or more of the following screening criteria or indices for suspecting a metabolic disorder: (1) a venous blood sugar of 130 mg./100 ml. or more 1 hour after the ingestion of 50 gm. glucose orally; (2) a history of diabetes in the immediate family; (3) a history of having borne a baby weighing 9 pounds or more (most of the literature refers to the "big baby" as weighing 10 pounds or more); and (4) a history of any 2 or more pregnancies complicated by fetal death, neonatal death, congenital anomaly, prematurity, or toxemia (albuminuria, hypertension and/or excessive weight gain).

All women who fail to meet any of the above screening criteria are said to "screen negative" and are called "negative screenees."

All women who are diagnosed or known to have diabetes before becoming pregnant are followed throughout their pregnancy, but do not constitute a part of the prediabetic research group under immediate investigation.

Within a week, if possible, after this screening process, all positive screenees are scheduled for a standard 100-gm. 3-hour oral glucose tolerance test, each woman being instructed carefully to eat 250 gm. of carbohydrate a day for 3 days before coming in for the test. The minimum carbohydrate preparation acceptable for the test is 150 gm./day.

In addition to the positive screenees, a random sample of women, regardless of screening results, are given a glucose tolerance test as candidates for negative control. Women meeting the screening criteria as well as the candidates for negative control who have a negative glucose tolerance test in one trimester receive repeat glucose tolerance tests in each subsequent trimester of pregnancy. If a candidate for negative control should be found to have an abnormal glucose tolerance test, the next case having a normal tolerance test is selected in chronological order of registration.

In this study carbohydrate metabolism is regarded as abnormal if any 2 or more blood sugar readings in the standard glucose tolerance test equal or exceed the following values expressed per 100 ml. of blood: 110 mg. fasting, 170 mg. at 1 hour, 120 mg. at 2 hours, and 110 mg. at 3 hours, all determinations being made by the Somogyi-Nelson method on venous blood.

These criteria for interpretation of abnormal carbohydrate metabolism during pregnancy were established in consultation with a committee of specialists in metabolic diseases. The classification of a research case is based solely on on the result of her glucose tolerance test in pregnancy.

All women with an abnormal glucose tolerance test who do not have frank diabetes and who have not reached the thirty-seventh week of pregnancy are selected for our research study. Every second woman in this group is selected for insulin therapy during the remainder of her pregnancy. Insulin dosage is the amount necessary to maintain normal glycemia throughout pregnancy. No insulin is given after delivery unless it is indicated because of evidence of clinical diabetes existing *post partum.*

During pregnancy, unless diabetes was known to have existed prior to conception, a woman is not considered to have frank, overt diabetes unless she has a blood sugar of 300 mg. or more or unless she has blood sugar findings approaching the 300 mg. level, together with classic symptoms of the disorder in the opinion of the internist.

Thus, there are three primary groups of women under careful observation and study: (1) women with abnormal glucose tolerance who receive insulin therapy during pregnancy (*insulin-treated*); (2) women with abnormal glucose tolerance for whom insulin is not prescribed (*positive controls*); and (3) women with normal glucose tolerance selected at random (*negative controls*).

There is no intervention with the progress of pregnancy, confinement, and delivery except when directed for purely obstetrical reasons by the obstetrician in charge.

The study includes (1) careful observation of mothers throughout their prenatal period; (2) good postnatal care and observation of both mother and child, including visits to the home by members of the Visiting Nurse Association; (3) physical examination of both mother and child, and a standard glucose tolerance test of the mother at 6 to 8 weeks *post partum*; (4) well-child care for the infant in the first year of life, including immunization and so on; (5) annual physical examinations and tolerance testing of all mothers for a period of at least 15 years; and (6) annual physical examinations and tolerance testing beginning at 4 years of age for all children for at least 15 years. Autopsy is done where possible in case of fetal or neonatal loss and maternal death.

Results

I present a few of our results, but in so doing I emphasize that this is a preliminary report; the study is still continuing. Any finding or conclusion at this time is a preliminary one and subject to change as more data are accumulated.

Through December 31, 1958, almost 17,000 pregnancies have been screened for detection of any suspicion of possible abnormal carbohydrate metabolism. About 42 per cent of them screened as positive.

Some analysis of data currently is available through December, 1957, and FIGURE 1 shows the screening results by various criteria through that date. At that time 11,352 delivered pregnancies had been screened; 44.4 per cent were positive screenees. This ratio between those suspected and those not suspected has not varied significantly at any time during the study.

It is noteworthy that 11.8 per cent of all pregnant women at the time of screening have a blood sugar of 130 mg./100 ml. or more 1 hour after taking 50 gm. of glucose; that is, they screened "positive to blood sugar." Of all pregnant women, 9.8 per cent have a history of having given birth to one or more babies weighing 9 pounds or more; 27.5 per cent of women had other abnormal pregnancy history, as has been defined earlier. Of all pregnant women, 11.3 per cent had a history of diabetes in the family. It should be remembered that all of these screening suspect groups overlap to some extent, and a woman may have 1 or any combination of the criteria.

TABLE 1 shows the results of the glucose tolerance tests by each screening criterion and the use of all criteria taken together. Here we find that of those women who would be suspected of having abnormal carbohydrate metabolism because of a positive screening blood sugar (from FIGURE 1 this would be 11.8 per cent of all the pregnant women), only 23.5 per cent actually had an abnormal glucose tolerance test, but this same single screening device yielded 44.9 per cent ("sensitivity" as seen in TABLE 1) of all the cases of abnormal tolerance tests and also had a relatively good specificity rate of 90.3 per cent (that is, gave 9.7 per cent false positive results). Of women with a history of a large baby, 13.5 per cent had abnormal glucose tolerance tests, as compared with 8.9 per cent of those women who had other positive pregnancy history and 11.5 per cent of the women who had a history of diabetes in the family.

If we consider all of those who may be suspected by at least 1 criterion, only 10.7 per cent would have an abnormal glucose tolerance test. This per-

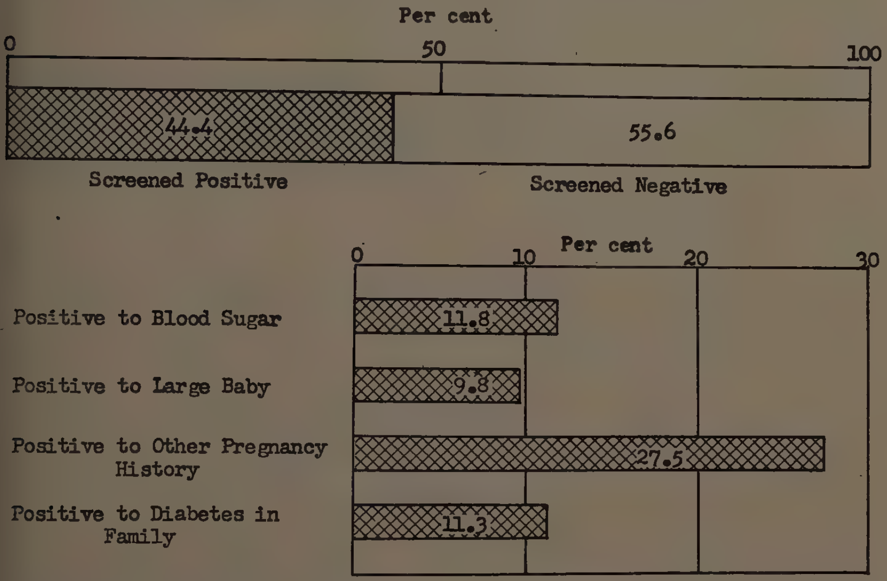

FIGURE 1. Pregnancy and the prediabetic state: screening results by various criteria from July 19, 1954 to December 31, 1957. A total of 11,352 pregnancies was screened.

TABLE 1

GLUCOSE TOLERANCE TEST RESULTS BY EACH SCREENING CRITERION

(July 19, 1954 to December 31, 1957)

Screening criteria	Glucose tolerance tests			Sensitivity rate	Specificity rate
	Number performed	Positive			
		Number	% of No. performed		
Positive to blood sugar	1085	255	23.5	44.9	90.3
Positive to large baby	907	122	13.5	21.5	90.8
Other positive pregnancy history	2482	221	8.9	38.9	73.6
Diabetes in family	1061	122	11.5	21.5	89.1
Positive to at least one criterion	4065	434	10.7	76.4	57.7
Negative to all criteria*	5082	134	2.6	—	—
Total screenees with GTTs	9147	568	6.2	—	—

* The actual number of GTTs performed on negative screenees is 378. Ten (2.6%) of these were positive on GTT. The number performed (5082) is an estimate of negative screenees expected to have a GTT based on the proportion of positive screenees having GTTs. The 2.6% rate is therefore applied to 5082 to estimate the expected number positive, that is 134.

centage seems small, but in fact does not differ very much from the percentage yield reported by Carrington *et al.*,[12] who, with a possibly more select group, found abnormalities of glucose tolerance tests in 111 of 790 patients so studied, or about 14 per cent.

Of particular interest in TABLE 1 are the specificity and sensitivity ratings of screening with at least 1 criterion. Screening "positive to at least one criterion" gives a sensitivity of 76.4 per cent; by this is meant that our present criteria are picking up about 76 per cent of those pregnant women who have an abnormal glucose tolerance test during pregnancy. However, we are missing about 24 per cent of those whom we would find if we gave such a test to all screenees instead of using the present procedure of screening and doing glucose tolerance tests on the positive screenees only. Also, we have a 57.7 per cent specificity with "at least one criterion positive," meaning that we currently must restudy about 42 per cent of all women who finally are classified as having negative glucose tolerance tests.

It is estimated that about 6.2 per cent of all screenees would have abnormal carbohydrate metabolism by our criteria if they were all given a glucose tolerance test. We performed glucose tolerance tests on a sample of 378 consecutive negative screenees and found that 2.6 per cent had positive results. Applying this percentage to all negative screenees who would have been expected to have a negative glucose tolerance test, we estimate that 134 would turn out to have a positive glucose tolerance test in spite of screening as normal in every way.

Our research patient case load through December 31, 1958 consists of 224 women who are insulin treated, 231 women who are positive controls, and 256 women who have been randomly selected as negative controls, or a total of 711 women being followed.

FIGURE 2 shows the percentage rates of abnormal outcomes of pregnancy for these 3 research groups. Although many of our research cases have given birth to several children, this bar diagram represents only the analysis of the outcome of first pregnancy under our observation. Abnormal outcome of pregnancy is defined as follows: live baby weighing 9 pounds or more at birth; live baby with congenital anomaly; baby alive but premature; or a perinatal death (including neonatal or fetal death).

A significantly greater number of total abnormal outcomes occurred among the positive controls than among the insulin-treated or negative controls. There appears to be no significant difference in the outcome of pregnancy, according to our criteria, between the insulin-treated and the negative control cases. Of particular significance is the greater number of live, big babies (9 lb. or more) born to the positive controls when compared with the insulin-treated or negative controls; the perinatal death difference is suggestive only. By our present criteria for congenital anomalies there is no appreciable difference among the 3 groups. No difference is seen in prematurity.

TABLE 2 shows the results of glucose tolerance tests in *post partum* follow-up of the first study pregnancies. All of 173 negative controls who were examined and given glucose tolerance tests some time within the 5 months after delivery were found to be normal. In the same type of examination about 92 per cent of both the insulin-treated and positive control cases had returned to normal, but a little more than 5 per cent in each of these groups still had some abnormality in their glucose tolerance tests. A little more than 2 per cent of both insulin-treated and positive control cases were revealed as diabetic by their first *post partum* glucose tolerance tests.* All or some of these 2 per cent could have been undetected diabetics prior to pregnancy.

With regard to our third specific objective of finding a practical procedure for identifying the pregnant woman who has abnormal carbohydrate metabolism or "prediabetes," the most encouraging results so far are those shown in TABLE 3. Here specificity and sensitivity rates for each hour of the glucose tolerance tests are presented. Of all the positive screenees who had abnormal glucose tolerance tests when first tested during pregnancy, 99.3 per cent had a 2-hour level of 120 or more mg./100 ml. blood. This level of sensitivity is satisfactory, but accompanying this good sensitivity is a specificity of only 91.4

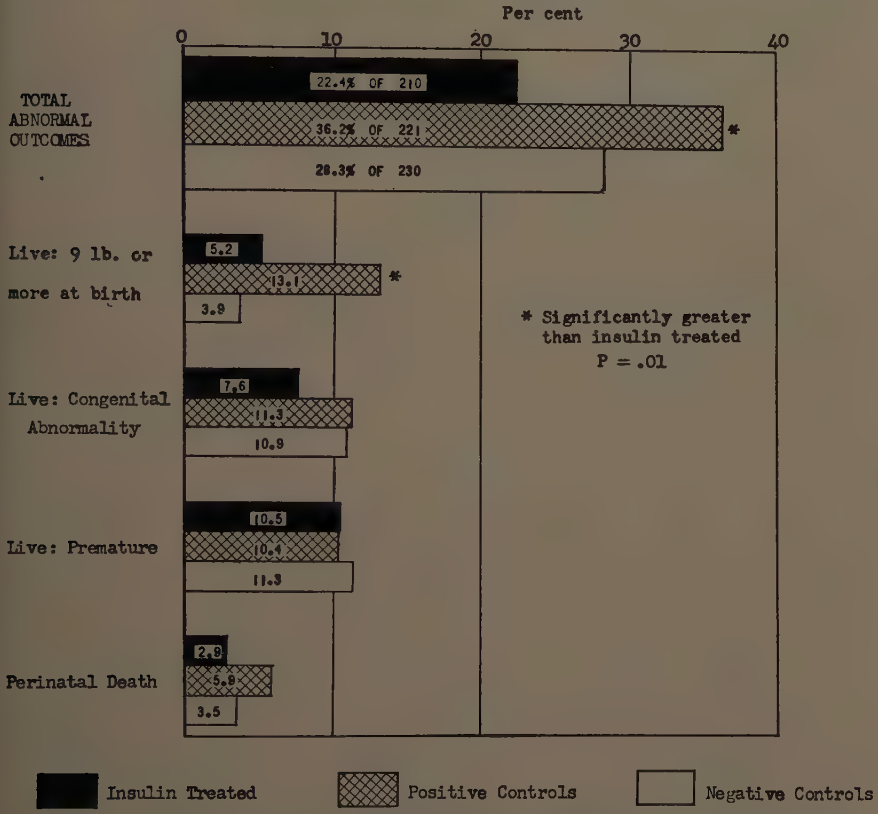

FIGURE 2. Rates of abnormal outcomes for insulin-treated patients and positive and negative controls through December 31, 1958.

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* Using the same blood sugar levels shown in our criteria for "abnormal carbohydrate metabolism in pregnancy," a glucose tolerance test outside of pregnancy is considered "diabetic" if any 3 successive levels are reached or exceeded or if both fasting and third-hour levels are equaled or exceeded.

per cent. This specificity rating means that 8.6 per cent of the positive screenees who had normal glucose tolerance tests unfortunately, apparently, had some degree of misleading elevation of blood sugar 2 hours after taking 100 gm. glucose.

The fact, however, that a single blood sugar analysis following 100 gm. of glucose in a standard glucose tolerance test procedure shows such a satisfactory ability to pick up all but 0.7 per cent of the abnormal glucose tolerance cases among those who screened positive encourages us to believe that a simplified and practical method of screening for abnormal carbohydrate metabolism in pregnancy may be forthcoming soon. First, however, we must do more studies on pregnant women who have screened entirely normal, that is, women who have normal-sized babies, no complications in pregnancy, and no diabetes in the family, (some even had a normal blood sugar by our criteria one hour after 50 gm. glucose) if we are to determine the sensitivity and specificity of the 2-hour postglucose level in all cases of pregnancy.

Table 2

Abnormal Carbohydrate Metabolism in Pregnancy

Results of Glucose Tolerance Tests in *Post Partum* Follow-Up of First Study Pregnancy Through December 31, 1958

Result through 5 mos. *post partum*	Negative controls		Abnormal CHO metabolism			
			Insulin treated		Positive controls	
	No.	Per cent	No.	Per cent	No.	Per cent
Total completed	173	100.0	180	100.0	191	100.0
Normal	173	100.0	166	92.2	177	92.7
Abnormal	—	—	10	5.6	10	5.2
Diagnosed diabetes	—	—	4	2.2	4	2.1

Table 3

Specificity and Sensitivity Rates of the Glucose Tolerance Test by Hour of Test*

(Using Somogyi-Nelson Method)

Hour	Blood sugar level	Specificity	Sensitivity
Fasting	110	99.9	6.1
First	170	99.5	37.8
Second	120	91.4	99.3
Third	110	96.7	82.3

* Based on 3908 GTTs made on women who screened positive through December 31, 1957. A test is considered positive when 2 hourly readings in a test equal or exceed the values shown.

On the basis of the analysis of the data collected so far, there are indications that we soon may be able to effect a considerable reduction in our laboratory work load without sacrificing any appreciable accuracy or efficiency. This means that we have reason to hope that we are close to a practical screening tool for abnormal carbohydrate metabolism or prediabetes in pregnancy.

Conclusions

Let me repeat that I have presented only some of the preliminary findings to date in a very extensive study that is still being continued. While there appears to be a significant finding of prevention of overweight in infants of so-called prediabetic women and a suggestion of reduction in perinatal mortality, the question of prevention of other complications such as congenital anomalies and toxemia is still unanswered. The possibility presents itself of ultimately evolving a practical program of detection of prediabetes in pregnant women that would be suitable for consideration by the medical profession for widespread application. It is emphasized, however, that recommendations for such a program must await important refinements needed and a clearer definition of the prediabetic state. Also, there must be more evidence of the actual significance, with respect to prevention, that can be attached to the detection of the prediabetic state in pregnancy.

Joslin *et al.*[13] have urged us "like Boulin, to stop saying that diabetes is irreversible and that the doctor has arrived too late." I agree heartily, but I do feel strongly that when the doctor does arrive he should be armed with all available facts about the value of early recognition of diabetes and the possible prevention of its complications and its progression to a permanent state. I hope that our research project ultimately will shed some clarification clinically and from the standpoint of public health on this confused state of so-called prediabetes in pregnancy.

Acknowledgments

All members of the Chronic Disease Program, Diabetes Field Research and Training, and the Prenatal Metabolism Research Staff have rendered most valuable assistance in the planning, operation, and evaluation of results of this research project.

Material assistance has been given by Ames Co., Inc., Elkhart, Ind., Eli Lilly and Company of Indianapolis, Ind., and E. R. Squibb & Sons, New York, N. Y.

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A CURRENT ESTIMATE OF THE PREVALENCE OF DIABETES MELLITUS IN THE UNITED STATES

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The size or importance of a disease as a public health problem is commonly determined by the number of persons who die of it, the number of persons who acquire it each year, the number of persons who have it, or the number of persons who become disabled because of it. Interrelationships among these statistics supply additional definitive information. Each of these measurements is deficient as a single gauge of the importance of a disease.

This discussion is limited to the prevalence of diabetes, which is the number of cases of diabetes in the population at a specific point in time. Limiting the discussion in this way should not obscure the fact that these other measures—incidence, disability, and mortality—are important. There would be far less concern about the number of cases of diabetes if it were not for the fact, for example, that diabetes is the eighth leading cause of death. The longevity of diabetics is still considerably below that of the general population, ranging from 17 fewer years of life among diabetic 10-year-olds to almost 4 years less among diabetics 70 years of age.[1] Diabetes is the third leading cause of blindness in the United States. One can undoubtedly add other statistics to indicate further the significance of diabetes as a public health problem, particularly from clinical observation of the day-to-day impact of diabetes on the lives of patients and their families.

Prevalence data will help focus the picture of diabetes as a public health problem. From such data come estimates of needs for physicians, nurses, nutritionists, and other personnel in caring for diabetics, needs for insulin and other medical supplies and equipment, and better definition of extraordinary needs in times of national emergency, as well as in ordinary situations, and the rationale for allocation of research funds. The estimate of unsuspected cases of diabetes unknown to patient or physician shows the need for case finding.

The need for such prevalence data has not gone unnoticed among investigators in the medical field. I shall not attempt to recount the studies conducted to obtain diabetes prevalence figures, but there have been many excellent studies; several of these are referred to in the course of this presentation. In spite of these various studies there is at this time no single figure on the prevalence of diabetes in the United States that would be entirely acceptable to all major groups interested in this field. There is no unequivocal national estimate based on adequate statistical methodology; therefore, any estimate of diabetes prevalence that could be presented is of necessity a crude working estimate based on scattered data and is verifiable only by comparison with local surveys and studies.

One might ask why, considering its importance as a health problem, there has not been a definitive study of the prevalence of diabetes. This same question can be posed for any disease because there has not been much practical development in the field of national prevalence data; however, I believe that major developments of this sort will take place in the next few years. Some of the reasons for the current lack of definitive information on diabetes prevalence may help us to appreciate the problems involved:

(1) A prevalence measurement depends for its accuracy on the precision of definition of whatever is to be measured. Therefore, a primary question is: What is to be considered diabetes for purposes of the prevalence measurement? Criteria for the definition of diabetes vary rather widely. There are large numbers of cases of diabetes that would meet even the most conservative diagnostic criteria. There are also many persons who would be called diabetics by some criteria but not by others, and there are even those referred to as prediabetics. This rather necessary ambiguity is reflected in estimates of prevalence.

In this connection it may readily be seen that changes in what is called diabetes will automatically produce parallel changes in prevalence estimates separate from any real trend in incidence or prevalence in the population. The evidence shows clearly that there has been a trend toward what may be considered as earlier recognition and diagnosis of diabetes. This trend seems destined to persist as research efforts continue to develop tests which, as Jerome Conn said in the 1958 Banting Memorial Lecture, "will be capable of detecting at an earlier age the great masses of people who are to make up our future diabetic populations."[2]

(2) Another reason for the lack of definitive information on disease prevalence is that the methodological problems of measurement were almost insurmountable until intensive study during the last five years brought forth some major breakthroughs. Even now, however, this is a very difficult area.

Various means have been attempted to uncover the prevalence of diabetes as known to the people themselves and to their physicians. The household survey on a sample basis is perhaps the most frequently used. In addition to variations resulting from the use of sampling, possible sources of error include: (a) purposeful withholding of information by the respondent, (b) his unwillingness to accept his doctor's diagnosis, and (c) confusion as to what the physician said. An example of the latter point would be the patient who was diagnosed as "renal diabetes" and reported simply "diabetes" to the survey interviewer.

The manner of measuring unsuspected diabetes (that is, disease unknown to the person or his physician) is even more difficult. It requires that a sample of the population be actually examined for diabetes. Ignoring for the moment the problem of definitive criteria for the diagnosis of diabetes, we find a greater source of error in the proportion accepting an examination of those in the selected sample. In the best studies to date, only about two thirds of the members of any sample population selected for study have actually accepted the examination. This is a serious source of bias. Reasons for nonresponse are many and varied, and they are currently the object of intensive study.

(3) Still another major factor, of course, is the cost of undertaking definitive nationwide studies of the prevalence of diabetes even on a sample basis. There is considerable question in the minds of most people as to whether at this time, or in the next few years, the improved accuracy of estimates of prevalence of diabetes and other diseases is worth the high cost involved. The cost problem

is not independent of methodology, and we may expect to see development of methods for more accurate prevalence estimates at a reasonable cost.

About 14 years ago the Public Health Service, Washington, D. C., became aware of the public health implications of the diabetes problem in the United States and the possibility of applying to diabetes problems many of the public health techniques that had proved so successful in controlling the communicable diseases. Early public health interest in diabetes led to investigation of the size of the problem and to estimates of prevalence. The need for and interest in such data are no less acute today than they were fourteen years ago.

Historically, the prevalence estimates made by the Chronic Disease Program of the Public Health Service have originated from the well-known study of Wilkerson and Krall in Oxford, Mass., in 1946 and 1947.[3] In the course of this pioneer study of prevalence in a typical United States community, over 70 per cent of the population of the town was examined for diabetes, using history and blood and urine sugar studies. A rate of 17.5 cases of diabetes per 1000 population was obtained. A few years after this study started several large-scale case-finding programs were conducted in which there was no attempt to study a population sample. On the basis of the Oxford study and these supplementary data, the prevalence of diabetes in the United States was estimated as being in excess of 2 million cases, approximately one-half of which were known cases and the remainder undiscovered and unsuspected. Clearly, this was a crude estimate based on a very minute population sample. Subsequent revisions have been made according to: (1) findings in other sample studies, (2) changes in population composition, (3) results from numerous large-scale case-finding programs, (4) special studies of the Chronic Disease Program and others, and (5) diabetes mortality data.

TABLE 1

ESTIMATED PREVALENCE OF DIABETES IN THE UNITED STATES IN 1958

	Number	Rate per 1000 population
Unsuspected diabetes	1,400,000	8.1
Known diabetes	1,500,000	8.8
Total diabetes	2,900,000	16.9

May I point out that the estimate refers only to the noninstitutionalized population; it accepts the prevailing diagnosis of known cases as being correct, and is based on conservative diagnostic criteria used to determine the unknown and unsuspected cases in the population. The Chronic Disease Program has endeavored to provide as reasonable and conservative an estimate as possible within the limitations of available data.

The current estimate of the prevalence of diabetes in the United States, then, is 2.9 million cases. Of that number, roughly 1.5 million cases are estimated to be known, and about 1.4 million are unsuspected cases (TABLE 1). These data produce rates of 16.9 per 1000 population in total, 8.8 known cases per 1000, and 8.1 unsuspected cases per 1000 population.

To our knowledge, there have been few studies reported in which an examination of any kind was given to obtain an estimate of the total prevalence of diabetes. While the criteria for diagnosis of previously unknown cases are not the same, the 3 studies shown in TABLE 2 provide some comparison with the Chronic Disease Program estimate. The Canadian study[4] rate is considerably less than that for the two eastern United States studies.[3, 5] Both of the United States studies have considerably higher rates than the national prevalence estimate and were conducted in areas having high diabetes mortality rates. From case-finding programs one also gets the impression that these areas have higher-than-average prevalence rates. This fact, of course, is discernible in the comparison of these 2 rates to the national estimate. While I am indebted to source material for the basic data on these various studies, the responsibility for rates and adjustments thereon is my own, and not that of my sources of data.

TABLE 2
DIABETES PREVALENCE IN STUDIES WITH PHYSICAL EXAMINATIONS COMPARED TO UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Year	Crude rate	Adjusted rate*
Oxford, Mass.	1946–1947	17.5	21.2
New Market, Ont.	1949	12.2	12.8
Baltimore, Md.	1953–1955	26.7	23.0
United States prevalence estimate	1958		16.9

* Adjusted according to the distribution of the 1957 population of United States by race and broad age groups.

TABLE 3
PREVALENCE OF KNOWN DIABETES IN SEVERAL STUDIES COMPARED WITH UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Year	Crude rate	Adjusted rate*
Oxford, Mass.	1946–1957	8.0	11.9
New Market, Ont.	1949	7.5	7.9
Baltimore, Md.	1953–1955	11.3	9.7
California Health Survey	1954–1955	5.5	5.5
Hagerstown, Md.	1955–1957	8.7	7.5
Kit Carson County, Colo.	1957	9.8	9.0
United States prevalence estimate	1958		8.8

* Adjusted according to the distribution of the 1957 United States population by race and broad age groups.

Comparative data on rates of known cases obtained in several surveys are shown in TABLE 3. In making these comparisons it should be observed that in addition to the usual sources of difference or error, there is the possibility that communities may differ considerably in the proportion of the total diabetes prevalence that is known to the patient or physician. A large-scale case-finding program may alter significantly the known case rate at the expense of the rate of unsuspected cases.

The methods used in the surveys shown vary widely and are worthy of comment. In the Oxford study previously referred to, histories were obtained on all persons examined, and the information was verified with the physician whenever a person said that he had diabetes.[3] The New Market, Ont., Canada, study was conducted in similar fashion;[4] more than 80 per cent of the population was tested. The data on Baltimore, Md., were obtained from the Study of the Prevalence of Chronic Illness conducted by the Commission on Chronic illness.[5] This study consisted basically of a household-interview survey of a random sample of the population followed by a "clinical evaluation" of a subsample of interviewees. This clinical evaluation obtained a response rate of about 63 per cent. As stated in the Commission's report, in reference to it, "The diagnosis of diabetes was based on clinical judgment taking into consideration history, physical findings, blood sugar and urine sugar determinations."

TABLE 4
PREVALENCE OF UNSUSPECTED DIABETES IN SEVERAL STUDIES COMPARED WITH UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Crude rate	Adjusted rate*
Oxford, Mass.	8.0	9.3
New Market, Ont.	4.8	4.9
Baltimore, Md.	15.5	13.3
United States prevalence estimate		8.1

* Adjusted according to the distribution of the 1957 United States population by race and broad age groups.

The California, Hagerstown, Md., and Kit Carson County, Colo. data were obtained by household-interview surveys of samples of the population.[6-8] Diagnosis of diabetes as reported by the respondent was not verified.

We see a wide range of rates in this group of studies. The result of the California Health Survey stands out as exceptionally low. The relationship between mortality and prevalence is by no means a sure one, but the mortality rate from diabetes in California is only two thirds that of the United States as a whole. It is therefore conceivable that the prevalence of known diabetes is considerably less in California than in the nation as a whole. The comparison between the current estimated national rate and the several studies is best shown by the race-age adjusted rates. These rates were obtained by applying the diabetes rates for specific race and age groups in the individual study populations to a standard population in order to eliminate differences caused solely by the varying composition in the study populations.

In comparing rates of unsuspected diabetes we are again limited to the few studies in which examinations of a sample of the population were actually undertaken; these are shown in TABLE 4. The current estimate for the United

States is again lower than that of the Oxford and Baltimore studies, but higher than that for the Canadian study.

A second source of crude comparative data on unsuspected diabetes is found in the results of case-finding programs conducted in various areas. The persons screened in these case-finding programs were not necessarily representative of the entire population in the area in which the programs were conducted. To the extent that these programs were successful in attracting high-prevalence groups in the population, their results were also less representative of prevalence in the general population. There have been 64 diabetes-screening programs with diagnostic information reported in the past several years to the Public Health Service.[9] In the average program 6 new cases of diabetes were discovered per 1000 persons tested, but unfortunately there is no breakdown of the results of these programs by age of persons tested. In most programs only a few persons under 25 years of age were tested. It is known that the older population, particularly those aged 65 years or more, are poorly represented in such programs. If the 2 under-represented groups, that is, the young very-low-prevalence group and the aged high-prevalence group, to a great extent balance each other, the rate of 6 previously unknown cases of diabetes per 1000 may be accepted as a crude measure of the yield of unsuspected cases.

According to a study conducted by the Public Health Service, the commonly used diabetes-screening techniques have a sensitivity of about two thirds. Thus, in a random screening of persons not in a fasting state 2 of 3 persons with unsuspected diabetes will screen positive in a screening test such as the Somogyi-Nelson at the 130-mg./100-ml. level on venous blood or its equivalent. This means that one of every 3 diabetics will be missed. These findings are based on conservative diagnostic criteria established by a committee of consultants including Arnold Lazarow, C. N. H. Long, F. D. W. Lukens, Alexander Marble, Henry T. Ricketts, Richard P. Stetson, and DeWitt Stetten, Jr. Therefore, the 6 cases per 1000 tested as found in case-finding programs represent only two thirds of the actual cases of unsuspected diabetes in the population. The estimated prevalence of unsuspected diabetes on this basis would be 9 per 1000 population, which is slightly higher than the 8.1 rate of our national estimate.

The future prospects are very good for more accurate and better-defined estimates of prevalence. The National Health Survey, a household-interview type of survey on a national sample basis, has been under way since July 1957. While no specific chronic disease prevalence data have been released, it is expected that a series of publications on specified diseases will be published in the near future. This will cast considerable light on the prevalence of known diabetes. In addition, a number of studies are in progress to improve methods of obtaining or estimating data on the prevalence of unsuspected disease. The increasing number of community case-finding programs should augment data on unsuspected diabetes.

As may be seen from the variety of rates that I have presented, it is most difficult to evaluate the accuracy of the present estimate of 2.9 million cases of diabetes. We believe it is a good, useful working estimate. Regardless of the precise figures used, the conclusion is inescapable that diabetes is an important individual and public health problem and that large numbers of unsuspected cases of diabetes await detection and medical care.

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DIABETES MELLITUS AND THE LIVING MICROVASCULAR SYSTEM

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Can anything be learned about the mechanism that damages blood vessels in diabetes mellitus by studying the dynamic morphology of the superficial microvascular system in man? The current opinion in regard to the answer to this question will be derived through a review of: (1) the preferential site for the study of the microvascular system, (2) the structures that can be visualized in this tissue, (3) the recording of the dynamic morphology, (4) the statistical validity of data derived from superficial microvascular systems, and (5) an analysis of the studies to date.

Preferential Site for the Study of the Microvascular System in Man

The tissues that can be considered practical for study are the skin, the mucous surfaces, the bulbar conjunctiva, and the retina. Of these tissues the bulbar conjunctiva is preferred, because the blood can be followed with ease as it passes through the numerous arteriolar-capillary-venous channels. Good definition of the circulation is obtainable because the vessels are superficial, parallel to the surface, and embedded in transparent connective tissue, and contrast is enhanced by the white background (the sclera). Also, this tissue is constantly irrigated by normal physiological fluid. Therefore, special preparation of the tissue is not required, and homeostasis is automatically maintained. The result of the anatomical and physiological constituents permit adequate optical magnification, a maximum of 225×, a resolution that is not permitted to a similar degree of ease by the retina, which is the only other tissue that can be considered for this type of study in man. Some of the inherent limitations in the study of the vascular and intravascular reactions of the retina at the microscopic level may be obviated or eliminated in the future by electronic image devices. The mucous surfaces and skin are not suitable because of their special vascular anatomy;[1] their terminal vessels are at right angles to the surface. It is difficult to differentiate not only the terminal portion of the arteriole but also the origin of the venule, since the capillary does not branch and there is no sharp morphologic differentiation among these vessels due to the limits imposed by magnification, lack of contrast, resolution, and orientation. Homeostasis of these vessels cannot be maintained because the intensity of the necessary illumination produces heat that can be compensated only by vasodilatation. In addition, these vessels are subjected to a chemical trauma produced by substances that must be used to make the overlying keratinized epithelium transparent.

Dynamic Morphology

When a microscope is focused on the bulbar conjunctiva, the component most readily visualized is the circulating blood. With the magnifications that are used most frequently (48 to 150×) the velocity of blood flow in health is too rapid in any vessel to distinguish cellular detail. At present there is no worthwhile method for measuring the velocity of flow in these vessels. The walls of blood vessels are vizualized, but with difficulty, because they can be seen only by reason of the differences in refractive index between blood, vessel wall, and sclera. These differences can be enhanced by manipulating the illuminating source but, at best, healthy vessel walls are difficult to distinguish from the supporting parenchyma. Therefore, in most instances the diameter that is measured is that of the flowing column of blood rather than that of the blood vessel. This error is least for the capillaries and venules, but it may become appreciable in arterioles when there is an anemia because of the increase in the peripheral plasma layer. The column of blood, however, does delineate accurately the configuration of the wall that in health are cones for arterioles and venules, with the smallest diameter of the cone at the junction with the capillary. The capillaries are cylinders. Cellular detail can be identified intravascularly whenever the velocity of flow decreases, which occurs in health when terminal arterioles constrict, when arteriovenous-anastomoses open (which decreases the flow rate distal to the shunt), and in short venules that connect adjacent venules and in which the pressures are nearly equal, which reduces or stops the flow in the connecting vessel. Under the above circumstances all of the formed elements of the blood remain single. Cellular aggregation (erythrocyte, leukocyte, or platelet) is absent, hemoconcentration does not occur, and leukocytes or platelets do not adhere to the inner wall of vessels. It has been stated that reduction of the linear velocity of blood flow per se produces erythrocyte aggregation.[2] This is not true in health. The blood flow through many areas of the body, especially in the liver, can stop for many minutes, and erythrocyte aggregation does not occur; however, they will form aggregates when the interfaces of the erythrocyte are abnormal. The slow flow does not produce abnormal interfaces per se, but only sets the stage for the formation of aggregates (for a discussion of how cellular aggregation is related to rouleau formation, see Bloch,[3] page 80). With normal blood flow and pressure, the normal number of intravascular cellular and plasma constituents, and a normal blood volume, a characteristic vessel pattern exists in the bulbar conjunctiva. The ratio in diameter between corresponding segments of arterioles and venules is 1:1.75 to 2.0. The vessels are not tortuous, and the pattern of distribution is regular. With a healthy intravascular and vascular physiology the connective tissue of the bulbar conjunctiva is transparent, permitting sharp focus of the circulating blood (this does not occur when there is a maintained reduction of blood flow through the microvascular system with associated exudation of plasma, whereupon the edema fluid causes increasing opacity of the connective tissue, with a concomitant decrease in the optical resolution). The morphology can be quantitatively determined with the use of a calibrated scale in the ocular of the microscope and a chronometer to measure the temporal events.[3]

Recording the Dynamic Morphology

If the observations are to be meaningful, the method of recording assumes importance. Difficulties exist. The microvascular physiologists have not agreed to any standard nomenclature or a consistent descriptive methodology, which makes it difficult to assess the results from different investigators (a detailed description of how to analyze and report the reactions has been given by Bloch[3]). The current vogue of illustrating the changes in this system by single photographs is of value for recording static changes in the vascular pattern,[4, 5] but such photographs are misleading in regard to the importance of the intravascular reactions and the dynamic changes of the vessels. One of the most important reasons for studying the living microvascular system is the dynamic response of blood vessels and the circulation. A common dynamic response is the behavior of cellular aggregates in the microvascular system. The behavior of such aggregates often has little to do with their size, but rather with their physical properties. For example, some erythrocyte aggregates are only slightly larger than a single cell, but can cause intermittent embolization of arterioles, a condition that is often not produced by aggregates three to four times larger. These erythrocyte aggregates are not illustrated in the static photograph, since the arterioles are usually small and deep in the tissue and the photographic contrast is poor. The ideal solution to this recording problem would be continuous motion-picture recording, but this is not possible due to technical difficulties, aside from the cost. The heart beat, respiration, fatigue due to eye fixation, and physiological nystagmus result in uncontrollable movement of the bulbar conjunctiva, which necessitates continuous refocusing of the camera while recording; this is not possible with standard cameras; consequently, this form of recording is at present of limited value. (Beam splitters cannot be used for this form of cinephotomicrography because the light intensity is insufficient. A special camera has been designed to overcome some of these limitations[6]). The best that can be done at present is to use a chart that lists the major components that are to be studied and, after recording these quantitatively place them in proper perspective by a descriptive summary.[3] To date only a few reports of this type have appeared.[7, 8]

Statistical Validity

Assuming that the dynamic morphology has been adequately reported, the question then arises whether these changes have other than local significance. The answer to this question comes, not from the study of the microvascular system in man, but from experimental animals ranging from amphibians to primates.[3, 9, 10] There are two parts to the question: (1) Does the blood in the superficial vessels represent a statistically valid sample of all of the circulating blood in the body? (2) Is this equally true for the vessels? From direct microscopic observations in more than 1000 animals (frogs, guinea pigs, cats, rats, mice, dogs, rabbits, and rhesus monkeys), in which the circulation on the surface of the body was made sequentially in the same animal (unanesthetized and anesthetized) and compared with the circulation in the organs of the thorax, abdominal cavity, and brain, it was found and concluded that, from the anatomical arrangement of blood vessels and from the mechanical mixing of the blood in the heart and lung, the consistency of the blood to the head (especially to the bulbar conjunctiva) is statistically the same as the arterial blood in the rest of the body. However, the consistency of the blood in the capillaries and venules in the bulbar conjunctiva is not necessarily a statistically valid sample. The sample will reflect statistical validity of connective tissue blood vessels similar to those that are not the site of active special metabolic processes of physiology or disease. For example, the physical composition of the blood is altered in capillaries and in the immediate post-capillary venules in tissues undergoing active metabolic processes such as muscular contraction or processes of localized disease. These processes can cause localized hemoconcentration or the formation of cellular aggregates that may not be reflected in the bulbar conjunctiva due to the dilution of these abnormalities by nonaffected blood flowing through adjacent tissues and diluted in the heart and lung by mixing. The arterioles in the bulbar conjunctiva or any other arteriole in the body contain a statistically valid sample of blood, while the blood in the capillaries and venules are statistically valid for the generic type of tissue (for example, connective tissue in the bulbar conjunctiva). The reactivity of the blood vessels will mirror the tissue type (for example, muscular, neural, hepatic, connective, respiratory, or intestinal.)[3]

Data Derived from the Bulbar Conjunctiva

To be able to evaluate the current studies it is worthwhile to review briefly the history of the microvascular system in man.[3] The study began more than one hundred years ago. Many of the early investigators noted cellular aggregation and abnormalities of vessel walls. Reports of microaneurysm have been described in diabetics for more than 50 years. Similar pathology was also observed in other diseases. Often the reports were conflicting or difficult to interpret, which led to a nihilistic attitude in regard to the usefulness of the bulbar conjunctiva to assess vascular damage.[11] This attitude has not been dispelled entirely.[12-14] In the past this attitude was justified, since the knowledge of what constituted the healthy microvascular system was meager. In respect to the dynamic morphology of this system, such an attitude can be maintained no longer. Relevant data for the system exist that have been obtained from many species and from many organs.[3, 15-26] The appearance and many reactions of the microvascular system are known precisely, which permits the recognition of pathology at its inception. Some of the current differences of opinion are not due to inaccurate data, but to inaccurate interpretation of them. There is a failure to understand clearly that a description of this system as pathological per se does not necessarily imply significance in respect to an alteration of function of the entire organism that can be detected necessarily by clinical symptoms or routine *in vitro* tests. Such symptoms and tests will become positive when sufficient microvascular pathology has occurred for a sufficient period of time. These parameters in general cannot be defined accurately at present. Description and significance should not be confused.

Before discussing the microvascular system in diabetes it is desirable to define in general an abnormal microvascular system and indicate some of the mechanisms that produce the abnormality. The discussion that follows is based on data derived by many methods and includes experimental animals where better optical resolution could be obtained and higher magnification (to 1270×) could be used.[3, 9, 27, 28]

By definition, the healthy microcirculation becomes pathological whenever an aggregate is present (erythrocyte, leukocyte, or platelet) and/or the viscosity of the plasma increases.[3] The aggregate, which is larger than the other cellular elements, changes the character of the flow. Independent or associated with aggregate formation is the adherence of leukocytes and/or platelets to the walls of postcapillary venules. Whenever the linear velocity of the flow is reduced, the first vessel walls that indicate dynamic morphologic abnormality are the postcapillary venules where hemoconcentration and dilatation occurs. These changes may be limited to a segment of one vessel and can occur at the site of the inception of disease. Recognizable disease processes can involve a large volume of blood and many microvascular systems. In man the erythrocytes are more commonly involved than the other formed elements. Therefore, with the continuation of the process that produced the formation of an aggregate, the majority of the circulating cells may become involved. At first the forces that bind the cells into aggregates will be weak, and the process occurs only in stationary or very slowly flowing blood, but finally the strength of the forces may be so strong that the aggregates are not fragmented in the arterial circulation. As a result of the increase in size and rigidity of the aggregates the velocity of blood flow decreases correspondingly.

The change from the healthy circulation is the degree of abnormality or "sludge" present at that moment.[3] With multiple observations it becomes possible to determine, not only the extent, but also the rate of the intravascular and vascular changes.

When an abnormal circulation is present:

(1) Either erythrocytes, platelets or leukocytes, or a mixture of such cells form aggregates.

(2) Leukocytes and platelets adhere to the endothelium of systemic blood vessels and, under special conditions, erythrocytes adhere to the leukocytes that line the vessel wall.

(3) In the presence of an increasing number of aggregates the normal laminar blood flow changes. At first there is an increase in the thickness of each lamina, followed by mixed flow that produces gross turbulences; finally, plug flow occurs where the aggregate is as wide as the vessel.

(4) The disturbance in the laminar flow produced by the aggregates results in a decreased velocity of blood flow. With the reduction in flow and the presence of aggregates, the homogeneous appearance of the healthy, flowing column of blood disappears, and the cellular aggregates become increasingly visible and sediment.

(5) Due to the decreased blood flow excessive plasma fluid is lost through the walls of venules.

As a result of such a circulation:

(6) The walls of blood vessels fail to receive adequate nourishment and the abluent properties of normal flow are altered, which results in a loss of their normal cone-shaped configuration.

(7) The walls of venules become increasingly permeable to the plasma that produced hemoconcentration, with associated local or general dilatation and sacculation and edema of the adjacent tissue.

(8) Finally, the number of circulating erythrocytes begin to decrease due to

the stranding of these cells in small blood vessels and presumably (indicated by direct observation in experimental animals) by phagocytosis in the liver.

In summary, the abnormal circulation is defined as the formation of aggregates by the formed elements of the circulating blood and/or an increase in the plasma viscosity that results in making the blood more difficult to circulate through the microvascular system, which can result in concomitant pathology of the vessel walls and a reduction in the circulating blood volume through thromboembolism, phagocytosis, and loss of plasma fluid.

A variety of abnormal processes can influence the microvascular system and, due to the limited number of responses of this system, morphologic similarities will exist. The differences of effect on the system will be determined by the metabolic disturbances of the disease. For example, cellular aggregation is the result of altered metabolism of the host due to a variety of noxious stimuli such as tissue trauma, antigen-antibody reactions, or bacterial or viral infections. Some of the factors that influence erythrocyte aggregation are: the chemical nature of the stimulus (physical trauma produces large rigid [20 to 40 μ] aggregates in contradistinction to a tuberculous infection where the aggregates may be small [10 to 13 μ] and soft); the duration of the stimulus (in trauma it is short and not repetitive while, in a tuberculous process, it can be active for months or years); the volume of tissue involved with patent blood flow through the infected area (in local trauma a very small volume of blood is involved while, in an extensive bacterial pneumonia, the volume is large); and the physiological status of the host (the aggregation of erythrocytes is less in malnutrition). Little work has been done to isolate the specific chemical inducers of such aggregation.[3, 29]

The above review illustrates some of the abnormal stimuli that can exist in disease, resulting in the production of intravascular cellular aggregation that produces pathology of vessel walls indicated by spasm, dilatation, constriction, or aneurysms. In general, each disease is limited by the damage it causes, which cannot be compensated for by the defense mechanisms of the host. Therefore, in a general manner, the dynamic intravascular and vascular reactions analyzed over the entire period of the disease will follow a rather consistent pattern; this has been documented.[3, 7, 8] However, no visible intravascular or vascular reaction per se is pathognomic, so that a diagnosis can be made only by observing the microvascular system.

Biomicroscopy of the human microvascular system of the bulbar conjunctiva began with Coccius.[30] Among the earlier investigators were: Donders,[31] who recognized erythrocyte aggregation; Bajardi,[32] who was interested primarily in reaction of the walls of blood vessels to drugs; Schleich,[33] who stressed the importance of measuring blood flow; Reimar,[11] who recognized that clumps of cells will sediment as flow decreases and described the effect of cellular aggregates on flow, but who recognized that these were important only when thrombosis occurred; and Luedde,[34] who clearly distinguished the difference of flow characteristics between arterioles and venules and biopsied the conjunctiva. The early reports of diabetes are those of Streiff,[35] who described granular flow and thrombi; Zeller,[36] who observed microaneurysms and invented a method for measuring the rate of blood flow; and Cesari,[36a] who correlated the changes seen in the circulation of the skin and conjunctiva and stressed the fact that these results were significant also for kidney and brain (also see Müller 1937–1939).[1]

The influence of intravascular erythrocyte aggregation on the microvascular system was stressed by the reports of Knisely *et al.*, which were followed by reports from other laboratories.[38] In 1951 Ditzel[37] began to report data obtained in patients with diabetes mellitus, using the bulbar conjunctiva method described by Knisely and Bloch. Ditzel and his associates have studied diabetic children,[39] normotensive diabetic adults,[40] and pregnant diabetics,[41] and have found that the vascular pattern is characterized by tortuosities and elongation of the capillaries, narrowing of the arterioles, dilatation of venules, and perivascular exudation leading to "hyaline" infiltration. These changes were associated with varying degrees of erythrocyte aggregation. Ditzel and his co-workers concluded that the venular distension and increased permeability are often maintained for long periods and, while these conditions are associated with cellular aggregation, they can be present when intravascular aggregation is minimal. The abnormal vascular pattern increases with the duration of the diabetes, and these are characteristic for the disease. The results of Ditzel and his co-workers are more than a corroboration of the earlier data for this disease, since they have used better criteria and better subjects. The finding of a specific reaction in the microvascular system in diabetes is not surprising, since "specific" patterns of reactions have been found in poliomyelitis,[7] myocardial infarction,[8] and tuberculosis.[3] In these diseases similarities and differences exist in the reaction of the microvascular system. This seeming paradox is explainable by the fact that only a few morphologic criteria are used. The dilatation, hypertrophy, and tortuosity of venules and concomitant edema are a common finding in another chronic disease, tuberculosis. It may well be worthwhile to compare the similarities and differences in the response of the bulbar conjunctiva to chronic reduction in blood flow to obtain an insight into the mechanism of how these diseases damage the vascular system. The studies of Ditzel *et al.*[37, 39-41] are commendable because they provide additional data about the morphologic dynamics of disease. More studies for a longer period of time are needed if the problems of chronic disease are to be elucidated. The data are still far too fragmentary. Not only are more studies required and for a longer period of time, but they should include quantitative results so that it will be possible to compare the data of different investigators.

If the morphologic mechanism of how diabetes affects the vascular system is to be known, it is necessary to study other vascular beds. The study of the retinal vessels is curtailed by the optical limitation of magnification, which is difficult to overcome. It may be possible to determine changes in the walls of venules, blood flow, and parenchyma with the present optics by using the technique of scanning microspectrophotometry.[42-45] This method shows great promise, but it is not yet perfected and, even if the promise is fulfilled, this technique will be insufficient. It is necessary to turn to experimental animals so that one may study the sites where the metabolic disturbances are originating or producing their major disturbances. Methods and information exist that make it possible to study the microvascular system in the liver and, to a lesser extent, in the pituitary, kidney, and pancreas, but such studies have not been made.

The question was proposed whether anything could be learned about the mechanism that damages blood vessels in diabetes mellitus by studying the dynamic morphology of the superficial vascular system in man. The question may be answered affirmatively because the blood flowing through the arterioles represents a statistically valid sample of all the arterial blood. The responses of the vessels in the bulbar conjunctiva represent those of connective tissue. Therefore, it is not possible from these data to predict with certainty how the microvascular systems of the pancreas, liver, pituitary, or kidney will respond. It is recommended that experimental animals be used to elucidate the responses of these organs and to obtain further data in man from the peripheral circulation.

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DIABETIC NEUROPATHY: EVALUATION OF FACTORS IN ONSET

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Although diabetic neuropathy has long been recognized, there are still many areas of inadequate knowledge and uncertainty. The diagnosis itself presents many pitfalls in view of the absence of any pathognomonic symptoms, signs, or laboratory findings,[1] as well as the lack of any specific pathological picture.[2] There is, however, one aspect on which there has been general agreement in the literature, namely, the background for the development of diabetic neuropathy. The accepted premise is that the neuropathy follows a prolonged period of poor diabetic control. Among the authorities subscribing to this viewpoint are Joslin,[3] Duncan,[4] Rundles,[5] Goodman,[6] and Martin.[7]

In reviewing our own series at the Mount Sinai Hospital I am unable to confirm this opinion, and many factors appear to cast doubt on the importance of control. The inconsistencies relating to neuropathy and diabetic control are manifest in several directions and include the following six points: (1) neuropathy may occur during good control; (2) there may be a simultaneous onset of neuropathy and the symptoms of uncontrolled glycosuria; (3) the neuropathy is unrelated to the duration or severity of the diabetes; (4) neuropathy may be the initial clinical manifestation of diabetes, unattended by symptoms of hyperglycemia and glycosuria; (5) the paradoxical precipitation of neuropathy following the institution of good control by diet, insulin, or tolbutamide has been observed; and (6) neuropathy may follow stress situations and in these instances, a relatively constant latent period exists.

(1) *Neuropathy during good control.* There is not infrequent reference in the literature to the occurrence of neuropathy in this clinical situation. The areflexia, pain, and paresthesia of diabetes commonly occurred in patients with diabetes under thorough control in the series of Woltman and Wilder.[8] Similar observations were made in three cases of neurogenic bladder[9] and in a series of patients under close supervision, good care, and good control.[10] Charcot's joints have been reported in well-controlled diabetes.[11] Other authors alluding to this set of circumstances include Broch and Klovstad,[12] Garland and Taverner,[13] De Jong,[14] and Jordan.[15] The last author stated that, since neuritis commonly occurs more often in the older person with mild diabetes than in the young one with severe diabetes, diabetic neuritis is not the result of hyperglycemia alone, and one must seek a more subtle factor.[16]

(2) *Simultaneous onset of neuropathy and diabetes.* This situation is well documented. The neuritic symptoms dominate the clinical picture, and the diabetic symptoms are elicited usually by a careful, routine history. This sequence of events was reported by Root and Rogers,[17] Bailey,[18] Epstein,[19] Boeck,[20] Broch and Klovstad,[12] and Jordan.[15] In our patients with this type of onset, no previous evidence or history of glycosuria could be demonstrated.

(3) *Neuropathy unrelated to duration or severity.* If poor control is the most important aspect in the pathogenesis of neuropathy, then severe diabetics (as measured by insulin requirement) and those with diabetes of long duration

should have the highest incidence of neuropathy. In our own experience there is no correlation between neuropathy and duration or severity of diabetes. Similar observations have been made by Jordan,[15] Rudy,[21] and Martin.[22] In fact, no good correlation has ever been made in any large series of patients.

(4) *Neuropathy as the initial clinical manifestation of diabetes.* This condition has been documented as the initial clinical manifestation of diabetes.[23, 24] These cases can hardly be ascribed to a prolonged period of poor diabetic control. The diabetes in these cases is diagnosed by finding an abnormal glucose tolerance test, carried out because of suggestive or compatible neurological syndromes and/or the presence of a family history of diabetes. Continued follow-up of these patients indicates that the overt diabetes makes itself manifest.

Similar observations have been recorded by Jordan,[15] Andrews,[25] Bailey,[18] Garland,[26] Sprague,[27] Muri,[28] Lincoff and Cogan,[29] and Broch and Klovstad.[12] My personal experience, as previously published, is in complete accord with this concept.

From the practical point of view, the diagnostic application of this observation may help solve some obscure clinical problems. The theoretical implication is that the neuropathy is a concomitant and not a complication of diabetes; this point will be discussed later.

(5) *The paradoxical precipitation of neuropathy following the institution of diabetic control.* This situation is incompatible with the postulate that the condition is due to a prolonged period of poor control. This observation has been sporadically reported in the literature.[5, 21, 27] In a study directed toward this set of circumstances, I have witnessed this phenomenon not infrequently in patients controlled with insulin,[30] as well as in those controlled with tolbutamide.[31]

The means whereby control is achieved are unrelated to the sequence of events, since similar phenomena are observed with diet alone, with insulin, or with tolbutamide. The neuropathy in this category follows a fairly constant latent period of 2 to 3 weeks of good control. A suggested explanation is that the sudden change in homeostasis results in a physiological aberration simulating a stress situation. Since the neurological syndromes are entirely similar to recognized forms of diabetic neuropathy, and since these syndromes clear in the face of continued use of insulin and tolbutamide, these drugs are exonerated from any intrinsic noxious role.[30, 31]

(6) *Stress situations leading to neuropathy.* Occurring incidentally in the course of diabetes such situations may lead to neuropathy independent of the state of diabetic control.[32]

Several authors have included this type of situation in their series including Rundles,[5] De Jong,[14] Rudy,[21] Epstein,[19] and Springer and Hymes.[33] I have observed 20 cases wherein the neuropathy followed the incidental occurrence of stress situations (TABLE I).

The resulting neuropathies are entirely characteristic of the recognized syndromes of neurological involvement as they occur in diabetes, and their clinical representations are independent of the nature of the stress. These facts indicate that the neuropathies are definitely part of the diabetic state. The stress factors that I have observed include abdominal surgery, prostatic surgery, amputations, cerebral vascular accidents, diabetic coma, barbiturate coma, acute myocardial infarction, acute infections, corticosteroid therapy, trauma, and the institution of control of the diabetes by diet, insulin, or tolbutamide.

Another characteristic of this group of patients is the presence of a fairly constant time interval or latent period. This latent period averages about 18 days, with a range from 7 to 30 days; its significance will be referred to later.

TABLE 1

Stress factor	Latent interval (days)
Surgery: prostate	24
Surgery: prostate	20
Surgery: amputation	10
Surgery: abdominal	10
Infection	14
Infection	21
Myocardial infarction	20
Myocardial infarction	24
Barbiturate coma	7
Corticosteroid therapy	23
Cerebral vascular accident	30
Control with insulin	7
Control with insulin	28
Control with insulin	14
Control with insulin	20
Control with insulin	21
Control with insulin	15
Control with tolbutamide	14
Control with tolbutamide	21
Control with tolbutamide	28
Average:	18.5

Table showing the latent interval of onset of neuropathy following the various stresses in 20 patients.

Discussion

That prolonged poor diabetic control may possibly lead to neuropathy is not denied. In addition, this paper is not meant to minimize the importance of good control in diabetes nor to enter into the controversial aspects of the merits of maintaining normoglycemia and aglycosuria in the diabetic patient; rather, the conclusions rest on the premise that neuropathy can and does occur independently of the presence, degree, or duration of hyperglycemia and glycosuria.

There has been an increasing awareness that diabetes is a complex, generalized, fundamental disease process of which the carbohydrate metabolic disorder represents a single facet. Other facets include pregnancy, microangiopathy (comprising retinopathy and nephropathy), and arteriosclerotic involvement of the larger vessels. All of the above may occur as the initial clinical manifestation of diabetes. Dry and Hines[34] felt that it would be correct to regard the vascular problem as a manifestation of an abiotrophy affecting the insulin-producing tissues and the vascular system. Recognition of this approach to diabetes has led to the performance of glucose tolerance tests and the concept of the existence of a prediabetic state.[35, 36] Root *et al.*[37] felt that diabetes mellitus is hereditary, but may remain latent until some stress brings it forth. This hypothesis carries with it a specific vulnerability of the central nervous and vascular systems. Thus, long before hyperglycemia and glycosuria are present, the patient may manifest other defective states.

From this point of view neuropathy emerges as an integral part of the variegated syndrome of diabetes mellitus. It is a concomitant, not a complication; it becomes a fundamental component of a multifaceted crystal and not an end result of failure of proper clinical management of disordered carbohydrate metabolism.

From the etiological consideration, no known factors have as yet been determined. In view of the varying backgrounds preceding the onset of neuropathy it would seem reasonable to conclude that there are probably several such factors. However, in those cases where the neuropathy follows stress situations, the evidence points to the presence of an operative toxic or metabolic factor. This assumption is based on the presence of a fairly constant time interval that is independent of the precipitating factor. It is directly comparable to the latent period of many known inciting factors that lead to various types of neuritis. These include postinoculation neuritis,[38] postvaccinal neuritis,[39] abortifacients,[40] arsenic,[41] and neuritis complicating penicillin therapy.[42] This parallel adds to the significance of our observations and suggests the general category of a sensitization phenomenon mediated via a toxic or metabolic factor that produces the cause-effect relationship.

It is worthy of comparative note that toxic drug manifestations resulting in intrahepatic cholestasis without neural involvement also have a similar latent period. These include arsphenamine,[43] thiouracil,[44] chlorpromazine,[45] chlorpropamide (unpublished observation), and metahexamide (personal communication from H. Dolger).

The presence of a latent time interval strengthens the validity of the contention that the stress factors are related to the neuropathy and are not a coincidental association. The fact that comparable situations with known toxic factors act in like fashion and express themselves similarly tends to support and corroborate this hypothesis.

Summary

The situations herein discussed that precede the onset of neuropathy are independent of the state of control of the diabetes. Hence, this negates the contention that a prolonged period of poor diabetic control is essential for the development of neuropathy.

Diabetic neuropathy should be regarded as a concomitant feature of the syndrome of diabetes mellitus rather than as a complication of the disease.

Awareness of the occurrence of neuropathy as the initial clinical manifestation of diabetes may help solve some obscure clinical problems.

The diverse character of the factors preceding the onset of diabetic neuropathy suggests that there may be several etiological determinants. However, the sequence of neuropathy following stress situations after a relatively constant latent time interval suggests the presence of an operative toxic or metabolic factor in this category.

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ACCEPTABILITY OF DIABETICS FOR LIFE INSURANCE

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Only about 30 per cent of persons with a known history of diabetes applying to the Metropolitan Life Insurance Company for life insurance are found to be acceptable, according to a recently conducted review of our experience. About two thirds of those persons who are offered insurance refuse the offer, thus leaving only 10 per cent of the original group of diabetic applicants who eventually receive insurance policies. A study in 1949 of its diabetic applicants by another insurance company showed an acceptance rate of about 40 per cent,[1] and a panel discussion in 1953[2] by medical directors of several insurance companies showed a range of about 30 to 40 per cent. In the past decade, therefore, despite our ever-increasing knowledge of the disease, the diabetic population has shown little improvement from an insurance-underwriting viewpoint. Why are 60 to 70 per cent of the diabetic applicants for insurance still found to be unacceptable for life insurance? What yardsticks are the insurance companies using in evaluating them?

Before the era of insulin therapy and for many years after insulin was discovered all diabetics were considered to be uninsurable. Gradually, however, the picture brightened, and in 1940 one company decided to insure diabetics on an experimental basis. Other companies soon followed this lead, and today almost every company that issues substandard-risk life insurance offers some type of coverage to a selected group of diabetics. It is an accepted fact, however, that even the most favorable group of diabetics cannot be considered for standard-risk insurance because, although their mortality rate has diminished, with a corresponding increase in life expectancy, it still is not as good as that of the general population. In general, insurance companies will decline applicants for whom the expected mortality is greater than 4 to 5 times that of standard risks. Substandard-risk insurance will be offered to the groups that have a mortality experience falling between the standard mortality limit and that for the declined group, the extra premium varying according to the amount of excess mortality. A study of the experience of the Joslin Clinic, Boston, Mass., for the period 1947 to 1951 on patients first treated at the clinic in the years 1930 through 1951[3] is depicted in FIGURE 1, the shaded area representing the difference in death rate between the diabetics and the general population. At age 15 the mortality rate per 1000 for diabetics was almost 6 times greater than that for the general population. At age 30 it was about 10 times greater, at age 40 about 4 times greater, at age 50 almost 3 times greater, and at age 60 about 2½ times greater. It is apparent, then, that diabetics decidedly fall into the substandard and declined classifications. Theoretically, all diabetics might be insured if additional classes of substandard insurance were devised to compensate for the added mortality that might be anticipated, but this idea is not practicable, because the cost for some groups would be prohibitive.

The premature development and relatively rapid progression of vascular and renal lesions is the prime reason for the high mortality among diabetics.

The over-all death rate from cardiovascular-renal disease is 2½ times greater in diabetics as compared to the general population. Analysis of the causes of death in recent years among diabetic patients,[3] as illustrated in FIGURE 2, shows that the diseases of the cardiovascular-renal system accounted for about three fourths of all deaths. Heart disease alone was responsible for nearly one half the total, and 2 of every 3 heart disease deaths were due to coronary artery disease. Next in order were cerebral vascular lesions, cancer, and renal disease. There is still some excess mortality among diabetics from infections, whereas death from diabetic coma and gangrene has decreased progressively.

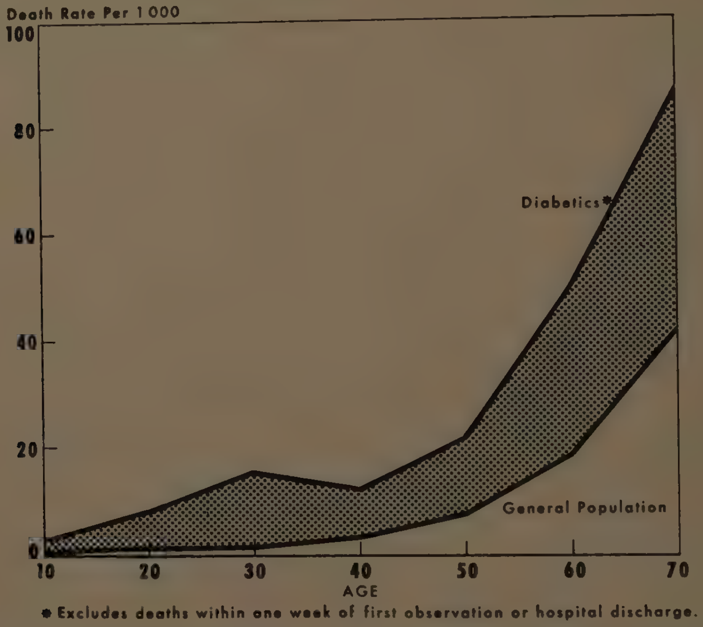

FIGURE 1. Death rate among diabetics and in the general population. The diabetic rate is that of patients of the Joslin Clinic in the years 1947 to 1951; the general rate is that of the white population of the United States in the years 1949 to 1951.

From an insurance-evaluation standpoint, there are two main groups to be considered: (1) persons with glycosuria, but without a specific history of diabetes, and (2) known diabetics. Concerning the first group, even before large-scale industrial health programs were begun, urine examinations in connection with life insurance probably represented the largest number of such examinations done on apparently healthy people. The discovery of numerous diabetics resulting from this has for many years been a major contribution of the life insurance industry to the field of diabetes detection.

Many studies have been made on the mortality of life insurance applicants with glycosuria.[4-8] It was found that those applicants who were limited to substandard insurance because of glycosuria had a mortality significantly above normal.[4-6] The amount of glycosuria, however, was not found to be a reliable index on which to base a substandard rating,[6, 9] and even the recent introduction of the enzymatic test, which is specific for glucose, is not likely to alter this. Prior to the introduction of readily available commercial testing materials of this type, the Metropolitan Life Insurance Company was using the enzyme test in its biochemical laboratory and had demonstrated its accuracy conclusively.[10] At the same time that this test was being evaluated, a study was carried out to determine if the results of a glucose tolerance test, usually performed on a different day, could be correlated with the initial amount of glycosuria.[11] The results were somewhat equivocal and confirmed the impression of Jimenis and his group, as reported in their study of glucose tolerance test results among persons showing glycosuria who applied to the Metropolitan Life Insurance Company from 1927 to 1944.[6] Some type of blood sugar test is therefore relied upon to help determine whether or not the applicant is a diabetic, but even these results have a limited value. An insurance company, by the nature of its business, can obtain only one test in almost all instances, and it has no control over the type of dietary intake of the applicant during the days preceding the test. Apart from this, the companies differ on test procedures and their interpretation: some companies use fasting blood sugars, others postprandial blood sugars, and still others use various interval values after glucose ingestion. The latter approach was adopted by the Metropolitan Life Insurance Company after a review (unpublished data) of two-hour glucose tolerance tests showed that the number of cases was so small where the fasting or half-hour blood sugar levels altered the interpretation of the test that it was deemed unnecessary to obtain the first two specimens. Granting, however, that variability in the type of test used and in the interpretation of the test exists, the question arises of how to handle the applicant who has a definitely abnormal level, say in the range of 130 to 150 mg. per cent on the 2-hour specimen, but who does not appear clinically to be a diabetic. Different companies have their own individual approaches to the problem and, until more extensive

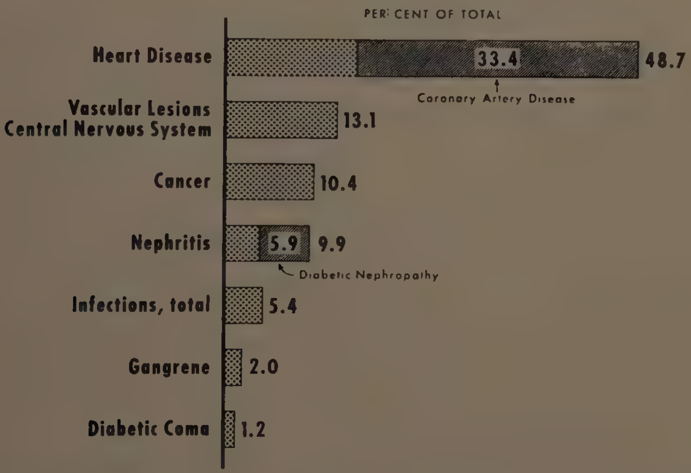

Figure 2. Selected causes of death among diabetic patients of the Joslin Clinic in the years 1950 to 1956.

mortality studies are carried out, this will continue to be an indefinite group. The mortality study carried out by Jimenis *et al.* on applicants who were given glucose tolerance tests did seem to indicate, however, that this group as a whole had a better mortality than the known diabetics. It also seemed to indicate that the mortality experience was less favorable in the group that had a grossly abnormal test as opposed to the group with a borderline or slightly abnormal response. Based on this experience, our approach at the Metropolitan Life Insurance Company has been to offer various substandard classifications according to the degree of abnormal response on the glucose tolerance test. We know that some persons in this group are diabetics but, within our pattern of operation, we cannot go ahead to establish the diagnosis. We are therefore dealing with the probabilities of a lesser or greater proportion of these applicants being diabetics, and our graded ratings are related to that. Analysis of mortality when we have a sufficiently large experience will indicate whether the ratings are correct.

The second broad group of applicants confronting the insurance underwriter comprises those applicants who have a definite history of diabetes. Numerous factors are taken into consideration in the medical evaluation of this group; four of these will be discussed.

First, there is the age of the applicant. Juvenile diabetics are still not considered to be insurable. There are ample mortality statistics to support this contention,[3, 12, 13] and most companies will not issue insurance to diabetics whose onset of disease occurs prior to age 15. At the other end of the spectrum an upper age limit of 55 is often set, but some companies have an upper age limit of 60, or even higher. This aspect of the problem will undoubtedly undergo re-evaluation in the near future because of the separation of diabetics into at least two major groups. One group has its onset of disease earlier in life, is usually insulin-deficient, and in many cases has a "brittle" type of diabetes. The second and major group consists mostly of obese persons with onset of disease in middle or later life. The difference between the two groups has been accentuated recently by the type of response to oral hypoglycemic agents.

A second factor is the duration of disease. According to one school of clinicians,[14] this is the only significant factor determining the onset of vascular complications. Prior to issuing insurance to diabetics, the Metropolitan Life Insurance Company studied the mortality data that had been collected over a period of years at the Joslin Clinic. TABLE 1 summarizes the results by age at entry to the clinic and duration of disease at first observation. The results are expressed in ratio percentage of actual deaths in the diabetic group to expected deaths in a standard population group. The company's contemporaneous mortality experience on standard risks was used as the basis of comparison in formulating the ratio. Bearing in mind that the limit of insurability for any impairment is now a death rate of 4 times that of the standard risk group (a ratio of 400 per cent), the data in TABLE 1 not only demonstrate an increasing death rate with duration of disease, but also show that diabetics have a death rate within the limits of insurability. TABLE 1 also shows a tendency for a lower mortality ratio in the older age groups. TABLE 2 represents the death rate per 1000 in the general diabetic population as seen at the Joslin Clinic on cases examined in the years 1940 to 1949 and traced to 1955. These data also tend to show an increasing mortality with duration of disease that is most apparent when comparing groups that are subdivided according to duration of disease at first observation. TABLE 2 represents only a sampling of the data and deals with patients aged 45 to 59 at the time of their first visit to the clinic. If, however, an applicant has had diabetes for a long period of time and is found to be free of significant complications at the time of application, he would usually be considered insurable.

TABLE 1
PER CENT RATIO OF ACTUAL TO EXPECTED DEATHS AMONG DIABETIC PATIENTS ACCORDING TO DURATION AT FIRST OBSERVATION
Cases With Insignificant or No Impairments

Age at observation (years)	Duration of diabetes at first observation	
	Less than 5 years (percentages)	5 Years or more (percentages)
25 to 34	329*	—
35 to 44	301	351
45 to 54	227	375
55 to 64	187	264

Experience of Joslin Clinic in 1939 to 1947 on patients first seen in 1930 to 1947. Expected deaths based upon Metropolitan Life mortality experience on standard risks.
* Based upon 7 deaths or less.

TABLE 2
DEATH RATES PER 1000 AMONG DIABETIC PATIENTS AGED 45 TO 59 YEARS AT FIRST OBSERVATION
Cases First Seen Less Than 1 Year and 5 Years or More After Onset

Duration from first observation	Duration of diabetes at first observation	
	Less than 1 year	5 Years or more
1 to 5 years	19.6	38.6
6 to 10 years	32.8	62.7
11 to 15 years	30.1	58.1

Experience of Joslin Clinic on cases examined in the years 1940 to 1949 and traced to 1955.

Third, control of the disease must be evaluated. In this regard the insurance company relies heavily on the statement of the applicant's attending physician. If control is inadequate or if the patient is not thought to be cooperative in carrying out treatment, he is unlikely to get insurance. More than any other disease or impairment, the total character, temperament, and surroundings of the diabetic must be taken into consideration when considering him for insurance. It helps if he has a good understanding of his disease and wants to cooperate with his physician in maintaining adequate treatment. If he uses alcohol to excess, he is likely not to be careful about adhering to the prescribed treatment and would not be considered a good risk. His type of occupation is also important. The experience of the Joslin group has for many years indicated that control is one of the most important factors, if not the one most important factor, determining when vascular complications will have their onset. Certainly, a great deal of stress is placed on control when an insurance underwriter attempts to assess the risk for a particular diabetic applicant.

Fourth, an attempt is made to estimate the severity of the disease. Some companies use insulin dosage as an indicator of severity, and 75 to 100 units of insulin daily is frequently set as a top limit for insurability. While insulin dosage is important, it is not a really good indicator of severity, and is not accepted as a hard-and-fast criterion of insurability. The recent advent of oral hypoglycemic agents for the treatment of diabetes has been watched with great interest by insurance companies and may influence underwriting practices in the future. A good indicator of severity is the presence of other impairments at the time of application. Complications of the disease such as retinitis, arteriosclerosis, or nephropathy, if present, mark the applicant as uninsurable within our present framework, and this rule is not likely to be modified in the near future. A recent mortality study[15] on insured diabetics tended to show that even moderate amounts of albuminuria, hypertension, or both, had a markedly adverse effect on mortality, whereas a moderate degree of obesity had much less of an influence. The marked effect that small amounts of albuminuria and/or hypertension had on the mortality would seem to indicate either that, without knowing it, diabetics who already had renal involvement

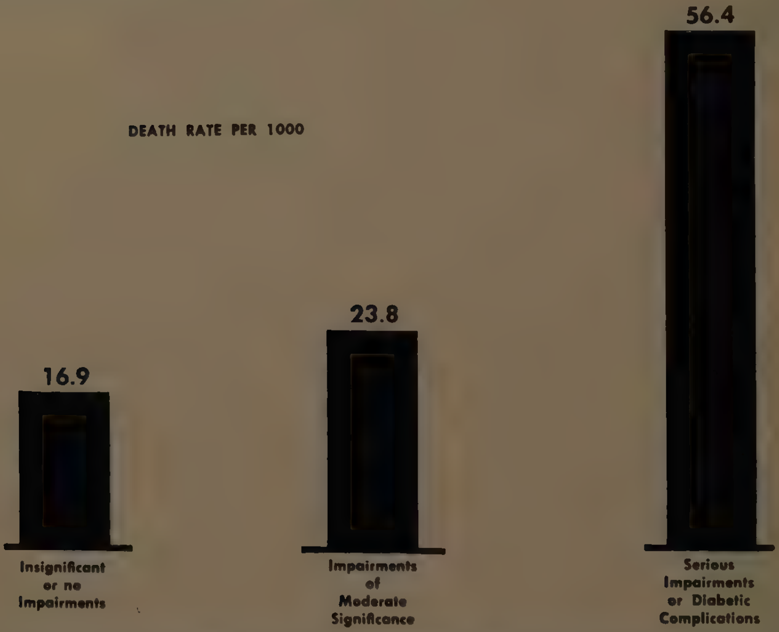

FIGURE 3. Death rate per 1000 of diabetic patients with and without significant impairments at first observation. These patients, aged between 15 and 64 years, were first seen in the years 1930 to 1945 (analysis made by the Metropolitan Life Insurance Company of Joslin Clinic records).

were insured, or that cases with early manifestations of renal disease went on to rapid termination. The Joslin Clinic experience, as represented in FIGURE 3, showed a greatly excessive death rate per 1000 in diabetics with significant impairments at first observation, as compared to the death rate in diabetics with insignificant impairments or none.

Taking all these factors into consideration, what has been the actual experience with diabetics who have been screened carefully and issued insurance? It is still too early to say accurately because of the limited material available to date, but preliminary studies[15, 16] show that, even in this carefully selected group, the mortality has been approximately three times that of standard insured risks. Since this is approximately the degree of elevated mortality that was expected, it would appear that the present methods used in the evaluation of diabetics have a sound basis. This is still an early experience, however, and because of the nature of the disease it is to be anticipated that the late experience will be less favorable. It is conceivable that some test or refined set of criteria may evolve that will predict prognosis with sufficient accuracy to allow an almost standard classification of certain diabetics. At any rate, it is hoped, that after more experience has been accumulated and analyzed, more diabetics will be considered insurable and broad subgroups will be delineated, so that the better risks can be given more favorable ratings.

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EMPLOYABILITY OF THE DIABETIC

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The most frequent question the newly detected adult diabetic requests of his physician is, "Will I be able to continue in my job?" The male patient wishes to know if the disease will interfere with his ability to work; the female becomes concerned about her job or her household responsibilities.

The physician must be able to answer these questions with accuracy and understanding. Usually the answers are simple, especially in the diabetic who is controlled by diet or by diet and an oral preparation. The patient who requires insulin for his control may present an employability problem if hypoglycemic reactions tend to endanger his own life or the lives of others.

The diabetic requiring insulin should never pilot an airplane or operate a moving crane. He should never be permitted to work near an excavation or drive a bus carrying passengers.

However, the diabetic should be assured that he is capable of supporting his family or of performing her usual household responsibilities. The diabetic child must be instructed early that he cannot be a jet pilot, but that he can do almost anything else. The best place for such instruction is the diabetic clinic or summer camp, where lectures on suitable employment are prescribed as part of an educational program.

Various reasons are given by employers for rejecting diabetics for employment. I shall demonstrate on the basis of surveys made in industry that education can remove most of the objections to the employment of the diabetic.

The five most frequent reasons given for rejecting diabetics for employment are: (1) insulin shock, (2) prolonged absenteeism, (3) increased compensation costs, (4) complications of diabetes, and (5) increased insurance costs.

(1) *Insulin shock.* Sudden unconsciousness may endanger the life of an employee, as well as the lives of others. For that reason proper job placement of the diabetic is important. For example, the Interstate Commerce Commission, Washington, D. C., records the case of a diabetic truck driver who required insulin. He neglected to inform his employer of his disease, and was not required to submit to a physical examination. He was employed as the driver of a large trailer truck operating across the country. One day, while driving on a mountain road, he lost consciousness, and his truck went out of control and struck several parked automobiles in a small town. This accident cost two lives, many injuries, and a great deal of property damage.

(2) *Prolonged absenteeism.* Employers fear the high cost of prolonged absenteeism because of its effect on production schedules, overtime costs, and disability insurance. A diabetic may develop tuberculosis or vascular disease and may be required to be absent for long periods of time.

(3) *Increased compensation costs.* Employers realize the possibility of prolonged disability following an industrial accident. A minor injury to a lower extremity may produce ulceration, gangrene, and possible amputation.

(4) *Complications of diabetes.* Complications, especially vascular disease,

may produce prolonged or permanent disability, which may be expensive to management.

(5) *Increased insurance costs.* This is frequently given as an objection to employing diabetics. However, this reason is reported to be unfounded, because group insurance costs are not increased by the inclusion of diabetics.

Some of these objections are valid, but may be removed with intelligent preplacement and periodic examinations, as well as careful control of the disease and proper job placement.

In a study of the diabetic in industry, Brandaleone and Friedman[1] reported the results of a questionnaire submitted by 63 companies representing 780,823 employees. The known incidence of diabetes among 286,662 employees of 39 companies reporting was 0.5 per cent. Forty-three of the 63 companies employed known diabetics; the remaining 20 did not. Thirty-three companies restricted the diabetics to nonhazardous work, 24 companies had no restrictions, and 6 had no set policy. Thirty-eight companies permitted an employee who developed diabetes to continue in his position, 24 companies restricted the type of work after diabetes was discovered, and 1 company reported no set policy.

TABLE 1*

DATA CONCERNING 40 KNOWN DIABETICS IN AN INDUSTRIAL POPULATION OF 3508

(Incidence of 1.1 Per Cent)

	Diabetics	All employees
Age	32 to 68 years	19 to 65 years
Average	54.5 years	46.1 years

Duration of employment since diagnosis of diabetes:
6 months to 22 years Average (3.9 years)

Diagnosis made by:

Medical department	18
Family physician	17
Unknown	5

* Data from Brandaleone and Friedman, 1953.[1]

TABLE 1 details information concerning 40 known diabetics for a period of 5 years. These 40 diabetics represented an incidence of 1.1 per cent in an industrial population of 3508 persons employed by a large urban transit system.

The age of the diabetics varied from 32 to 68 years, and averaged 54.5 years. The age of all employees varied from 19 to 65 years, and averaged 46.1 years. Four persons had diabetes of several years' duration prior to employment. The duration of employment after discovery of diabetes averaged 3.9 years, varying from 6 months to 22 years. Of the total number of 3508 employees, 3252 were male and 256 female; there were 1873 operators, 953 mechanics, 336 clerical workers, and 346 in miscellaneous jobs. Of this group, diabetes was present in 17 bus operators, 10 mechanics, 4 office workers, and 9 miscellaneous workers (such as stockmen, laborers, elevator operators, and superintendents). The diagnosis of diabetes was made by the company medical department in 18 cases, by private physicians in 17 cases, and by other, unknown, sources in 5 cases.

Eleven employees took insulin regularly. Sixteen took no insulin, and 6 took insulin irregularly. In the remaining cases no data were available.

Applicants with diabetes requiring treatment with insulin were not rejected except for the position of bus operator. For such persons, employment in other work was recommended.

Diabetic control was classified in four categories: (1) good, (2) fair, (3) poor, and (4) unknown. A person was considered under good control when he maintained his body weight and had minimal glycosuria and no acetonuria. Fair control was signified by moderate to severe glycosuria, maintenance of body weight, and absence of acetonuria. Control was considered poor when the person lost weight and had severe glycosuria and intermittent acetonuria.

Seven patients maintained good control, 6 were fair, and 3 were poor; in 24 the status was undetermined. Seven of these 24 are no longer with the company. The remaining 17 (43 per cent of the total number of diabetics) did not take advantage of the opportunity to cooperate with the medical department.

Twenty-seven employees were able to continue in their jobs. Five were permitted to continue as bus operators when they were not taking insulin. One patient who had pulmonary tuberculosis was unable to work because of it.

TABLE 2*

ABSENTEEISM IN A TRANSIT SYSTEM: FEBRUARY TO OCTOBER 1951
(Number of days absent per employee per year)

	Sickness	Accident	Nonmedical
Total group	11.81	0.69	7.10
Diabetics	26.2	0.80	4.60

* Data from Brandaleone and Friedman, 1953.[1]

In an effort to evaluate the efficiency of the diabetic in industry the three following factors were studied: (1) ability to work, (2) absenteeism and (3) caliber of work as defined by accident rate.

(1) *Ability to work.* Twenty-seven employees were able to continue in their jobs. Five were permitted to continue as bus operators when they were not taking insulin. One patient who had pulmonary tuberculosis was unable to work because of it. Five patients were retired on reaching the prescribed age limit. Two resigned for reasons unrelated to diabetes. All of these patients were employed at the time of this study.

(2) *Absenteeism.* A meticulous study was made of absenteeism in a large transit system for the 9-month period from February to October 1951. Absenteeism was divided into 3 categories: illness, accident, and nonmedical. Five of the 40 diabetics were not employed during this period of study, having been pensioned previously. The remaining 35 were compared to the total group, and the results are shown in TABLE 2.

The absence for sickness of the diabetic group requires a word of explanation. Two individuals were absent for long periods of time: one patient with pulmonary tuberculosis was absent the entire 9-month period of study because of the tuberculosis and not because of his diabetes. The other was absent for 146 of the 273 days because of an ulcer on the foot. While these 2 patients represent only 6 per cent of the diabetics, they were responsible for 61 per cent of the number of days absent with illness of the diabetic group.

The time off for illness for the other 33 diabetics (94 per cent of the cases) was 10.8 days per employee per year. This is approximately the same illness absentee rate as for the nondiabetic group.

(3) *Caliber of work as defined by accident rate.* An effort was made to compare the caliber of work, as measured by the accident rate, performed by diabetic and nondiabetic bus operators.

Thirteen of the 17 diabetic bus operators drove in routes of the city of New York. The statistics for the diabetics as compared to those for the total group are shown in TABLE 3.

Although, from a statistical viewpoint, the number in the diabetic group is deemed insufficient to permit a conclusion, the data for the diabetic group seem not far removed from those for the nondiabetics. Furthermore, both sets seem to show a sharp decrease in accidents after the fourth year of operation.

TABLE 3*

NUMBER OF ACCIDENTS PER MAN IN 2-YEAR PERIOD FROM JULY 1, 1950 to JUNE 30, 1952

	300 non-diabetic operators	Diabetic operators	Number of cases
First 2 years of driving	5.8		0
Third year of driving	5.2		0
Fourth year of driving	5.3	5.6	6
Fifth year of driving	2.7	3.6	5
Sixth year of driving	3.3		
Seventh year of driving	3.2	3.0	2
			13

* Data from Brandaleone and Friedman, 1953.[1]

Another study was made by the Committee of Employment of the American Diabetes Association, Inc., New York, N. Y.[2] In response to questionnaires, 127 companies employing approximately 1,900,000 workers, including 2658 diabetics, presented the following data (TABLE 4):

Although 123 companies (97 per cent) perform preplacement physical examinations, only 106 of them (83 per cent) include a routine urine examination.

Eighty-eight companies (69 per cent of the total of 127) employ known diabetics, while 31 (25 per cent) do not; 8 companies (6 per cent) did not answer.

Of the 31 companies that do not employ known diabetics, 17 based their policy on "previous poor experience"; 16 on "insurance reasons"; 4 on "statistics indicating unreliability"; and 5 on "other reasons." Eleven gave more than 1 reason, as the totals indicate.

Companies employing known diabetics rated their "experience as to reliability and absenteeism" as follows: "satisfactory," 65 companies (51 per cent); "same as average worker," 60 companies (47 per cent); and "unsatisfactory," 7 (6 per cent). Of these companies, 44 gave more than 1 answer.

A total of 110 companies (87 per cent) indicated that their employees participated in insurance and other employee benefits. The major conclusion drawn from these data was that "large companies have a more enlightened approach to the subject."

In a recent publication, Wade[3] reported a study of 266 diabetics in an employee population of 20,466 (an incidence of 1.3 per cent). This group of diabetics had an average age of 42.6 years. Seventy-five per cent of the cases were discovered after 40 years of age. Work assignments had usually become stabilized and were not incompatible with the diabetic state. In a very few instances the advent of diabetes was the cause for job reassignment.

TABLE 4*

RESPONSE TO A QUESTIONNAIRE SUBMITTED TO INDUSTRY

1. Number of employees		1,871,640	
2. Number of diabetics		2,658	
3. Do you do routine preplacement physical examinations?	Yes:	123	97
	No:	3	2
	Did not answer:	1	1
4. Does this include a routine urine examination?	Yes:	106	83
	No:	21	17
5. Do you employ known diabetics?	Yes:	88	69
	No:	31	25
	Did not answer:	8	6
A. If not, is it based on:			
(1) Previous poor experience?		17	13
(2) Insurance reasons?		16	13
(3) Statistics indicating unreliability?		4	3
(4) Other reasons?		5	4
Note: 11 gave more than one answer		11	9
B. If you do, what is your experience as to reliability and absenteeism:			
(1) Satisfactory?		65	51
(2) Unsatisfactory?		7	6
(3) Same as average worker?		60	47
Note: 44 gave more than one answer		44	35
(4) Do your diabetic employees participate in insurance and other employee benefits?	Yes:	110	87
	No:	0	0
	Did not answer:	17	13
(5) Do you make any concessions as to rotation of shifts?	Yes:	35	28
	No:	68	53
	Did not answer:	24	19

* Modified from: Committee on Employment of the American Diabetes Association.[2]

Wade commented that the workers continued satisfactorily in both sedentary jobs and heavy physical labor.[3] Approximately 10 per cent of the total group of 266 diabetics was restricted, but most restrictions were imposed for cardiovascular-renal disease rather than for diabetes; these restrictions were the same in other workers with similar vascular disease without diabetes. Restriction of work assignment was sometimes necessitated by concomitant vascular disease. Only 10 workers (3.2 per cent) were restricted because of their diabetes alone.

The possibility of insulin reactions required the reassignment of 8 of these 10 workers to jobs where they were not likely to injure themselves or others. The other 2 diabetics who were doing shift work could not be adequately controlled because of the irregularities of their working hours.

Much of the data presented by Wade demonstrated the importance of an adequate occupational health program.[3] Early detection of asymptomatic cases was most frequently the result of periodic health examinations. Proper education of the diabetic is of vital importance.

Twenty nine and eight tenths per cent of all the diabetics had cardiovascular renal disease, while 31.1 per cent of them were suffering from obesity. It was Wade's opinion that diabetics should not be hired where normal advancement requires rotation through assignments necessitating the operation of motor vehicles or fast-moving or heavy machinery, nor should shift work be essential in the course of their careers.[3] Failure to assume a fair share of the "graveyard" shift is not looked upon favorably by fellow workers. The desire of most shift workers to rotate at relatively short intervals makes an adjustment of eating and insulin schedules complex and incompatible with optimal diabetic control.

On the other hand, diabetes does not preclude productive employment, as evidenced by the experience reported in Wade's study.

Improvement in the employment of the diabetic depends on the education of: (1) the diabetic himself, (2) the family physician, (3) industrial management, and (4) the industrial physican. The use of oral preparations has removed most of the objections of industry. However, an understanding of the problems of the diabetic will reduce further any existing objections to employing diabetics.

The diabetic must understand his limitations in industry if he requires insulin or suffers with complications of his disease. He must be taught that proper care of his disease, including close cooperation with his family and industrial physicians, is the best way to insure his ability to continue in his work.

The American Diabetes Association states that the poorly controlled, uncooperative diabetic should be refused employment, while the well-controlled, cooperative diabetic is a good employment risk.

The attending physician should understand the type of work performed by his patient so that he may be able to control the patient's disease. In addition, the patient's physician should be in communication with the industrial physician, who better understands the problems on the job.

The education of management in industry is most important. The studies of the Committee of Employment of the American Diabetes Association have shown that larger industrial organizations having medical departments were more enlightened in the problems of employing diabetics and were not concerned about employing well-controlled diabetics who were supervised periodically by both the family and the industrial physician.

The results of studies in industry show that the diabetic may be compared to the nondiabetic in his ability to work, the caliber of work, and his absentee record, except in a few isolated instances.

The industrial physician is an important factor in the care of the diabetic in industry. This practitioner must be alert to the condition of the employee, the accuracy of his control, and his ability to do his job; he must also be able to recommend proper job placement. He must supervise the diabetic and be sure that the employee has periodic supervision by his own physician. Close supervision and cooperation will help prevent the complications of the disease or indicate early the presence of a complication for prompt therapy.

The industrial physician must always be alert to the development of diabetes in an employee and recommend the employee for prompt and proper therapy by his family physician.

The diabetic seeking employment should submit to a careful preplacement examination and, later, to regular periodic examinations to assure adequate diabetic control and the absence of complications. On the basis of preplacement examinations, proper job placement may be made.

On the basis of a more enlightened attitude, the objections to employing diabetics are minimized to negligible proportions.

A more liberal attitude in the employment of diabetics may be seen from a recent report of the United States Civil Service Commission, Washington, D. C., which has published the pamphlet *Employment of Diabetics in Federal Service*,[4] which states:

"The U. S. Civil Service Commission believes that persons with controlled diabetes may be good employees and that it is good business to hire them. Regardless of this condition a diabetic is capable of safe and efficient service in an appropriate job, provided satisfactory control of his condition is maintained.

"Mild diabetics who require no insulin for the control of their metabolic disturbance present no special difficulty in regard to placement. They are ordinarily capable of performing any type of work for which they are otherwise qualified.

"More severe diabetics who require insulin and a regulated diet are acceptable for appointment to many positions, including arduous-duty positions provided medical evidence shows the condition is under control. When medical evidence is furnished to show the condition is properly controlled, the Commission will accept the individual's application for consideration for positions which do not require work at heights or around dangerous power-driven machinery, work involving the operation of motor vehicles, or work in any other environmental situations which would render the applicant a hazard to himself or others in the event he experienced a lapse of consciousness while on duty. Some individuals with less severe and properly controlled diabetes are given favorable consideration for motor vehicle operator positions, particularly those involving incidental driving.

"More severe diabetics who require insulin and a regulated diet but who are uncooperative and/or poorly regulated are not recommended for Federal employment. There is a small group of unstable diabetics who are cooperative and are under adequate medical supervision, but in whom it is difficult to maintain normal standards of control. These individuals are not denied employment if their conditions will permit employment in the position for which application is made.

"Diabetics should carry cards or tags at all times identifying their condition.

"Diabetics should be under the guidance of their clinic or personal physician and should report periodically for physical examination and medical evaluation."

Summary

Education of the diabetic patient, of the family and industrial physician, and of industrial management will lead to a better understanding of the diabetic employee.

If the diabetic is well controlled and cooperative and presents himself for periodic examinations, he may be useful to his employer and to himself.

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Part II. Recent Advances in Studies of Insulin Secretion and Certain Metabolic Effects

ISLET CELL FUNCTION

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The islets of Langerhans are small masses of specialized cells lying in the external secreting tissue of the pancreas. In the rat, they make up about 1 per cent of the total pancreas and, if all the insulin is assumed to be in the islets, then the insulin content per gram of islet tissue is about 300 U./gm.; that is, somewhat more than 1 per cent of the islet is insulin.

The islets make and secrete insulin. The evidence for this is good, and has been reviewed elsewhere.[1] The beta cells appear to be the structures responsible for the insulin of the pancreas, since there is a close parallelism between the insulin content of the pancreas and the granulation of the beta cells when they are stained with aldehyde-fuchsin stain. I shall limit this discussion to a consideration of the function of beta cells.

The changes in the histological appearance of the beta islet cells may be gross morphologic alterations, as one might observe with hydropic degeneration or extensive atrophy or disappearance of beta cells or changes in the degree of granulation of their cytoplasm when stained in a particular way. In addition, changes concerned with the Golgi apparatus may help in an assessment of islet cell activity. The strictly histological indices are considered by others elsewhere in this monograph.

In some species there is good correlation between the degree of granulation of the beta cells and the insulin content of the pancreas. Granulation studies therefore give somewhat the same type of information as studies of the insulin content of the pancreas. Because of this, we shall consider these two types of investigations together. The insulin content of the pancreas and the state of granulation of the islet cells, by themselves, tell little regarding islet activity but, taken in conjunction with other measurements or taken under different experimental conditions, this content may give a great deal of valuable information concerning islet function. A reduction in the insulin content of pancreas or in the granulation of islet cells may result from some disproportion between the production and secretion of insulin (FIGURE 1). This effect may be due to an increased secretion of insulin that is out of proportion to its production, or to a decreased production of insulin that is out of proportion to its secretion. The disproportion accounts for the change, but it is conceivable that a level may be maintained despite changes in secretion if secretion and production remain balanced. Thus, sudden changes in secretion or excessive changes would be more likely to cause changes in the insulin content of pancreas or in the granulation of islet cells than slow changes. With slower changes, it seems possible that production could keep pace with secretion and that, accordingly, neither islet granulation nor insulin content need change.

In any discussion of the regulation of islet function, it is necessary to consider both insulin production and insulin secretion. It is also well to appreciate that the same factors may not influence primarily both production and secretion, and that the mechanisms involved in their regulation may be different.

There seems to be a limit to the concentration of insulin within islet cells, and an increase in the insulin content of pancreas above a certain amount thus comes as a result of new islet cell formation. Below this level, changes in the concentration of insulin per islet cell can occur. Hence, an additional factor may influence the insulin content of the pancreas and, conceivably, the granulation of islet cells. This is the total volume of the islets. If the total amount of islet tissue increases, then the insulin content of the pancreas increases, provided that the concentration per cell remains the same. If the total insulin content of the pancreas remains the same, but the total volume of islet tissue is increased, then the concentration per cell will be reduced, and the granulation of the cell also will be reduced. Changes in the amount of acinar tissue can greatly influence the concentration of insulin in the pancreas as a whole, but should not affect the total insulin content of the gland.

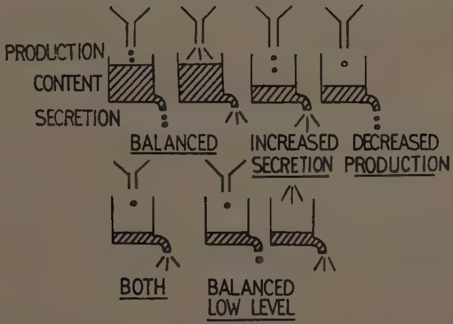

FIGURE 1. Diagrams to indicate the relation between the insulin content of pancreas production of insulin, and secretion of insulin. The diverging lines represent greatly increased secretion or production. The content falls when the disproportion between production of insulin and secretion of insulin is such that secretion exceeds production. The content rises when production exceeds secretion. The level stays at one point when production and secretion are balanced.

The endocrine pancreas has several functions, including the production, storage (limited), and secretion of insulin and growth or regeneration. Since diabetes is a condition resulting from a relative lack of insulin, I shall deal chiefly with those things that influence secretion and the growth of the islets. Unfortunately, the secretion of the islet cells cannot be collected and measured directly, as can the secretion of the exocrin glands. However, some information about the secretion of insulin can be obtained indirectly by noting histological changes in the islet cells or by measuring changes in the insulin content of the pancreas or in the insulin that has entered the blood.

If the production of insulin by the pancreas does not change, then estimating the level of pancreatic insulin provides a measure of secretion. Secretion may be estimated also by measuring the changes in the level of insulin in the blood. This blood level of insulin depends on the balance between the secretion and removal of insulin, as shown in FIGURE 2. The reservations are similar to those mentioned for the insulin content of the pancreas. However, at present it is easier to investigate the relationship between the production and secretion of insulin in the pancreas than between the secretion and removal of insulin from blood. This removal must also involve renal excretion, which further complicates the picture. The insulin content of peripheral blood by itself, therefore, does not provide a very satisfactory index of insulin secretion.

A better indication of insulin secretion would be the total insulin content of the blood leaving the pancreas. The islets constitute about 1 per cent of the pancreas, and the circulation to the islets is not separate. Since one cannot separate the blood flow to the islets from that to the acinar tissue and, also, since fluid may be removed from blood as it passes through the pancreas, it is necessary to know the amount of blood leaving the pancreas and the insulin contained therein. If it were possible to measure the total amount of insulin leaving the pancreas, then changes in this value would satisfactorily indicate changes in insulin secretion, provided there were no differences in the amounts of insulin held by the other tissues of the pancreas. However, any procedure permitting such measurements must be done acutely in anesthetized animals. The operative procedures and the anesthetic must produce changes in blood flow and changes in function that are not easy to compute. Not the least of the difficulties in this and in the previous method, as well as in the one to follow, is the accurate quantitative measurement of the insulin content of the blood. The difficulties in estimating this are well known and will not be discussed here.

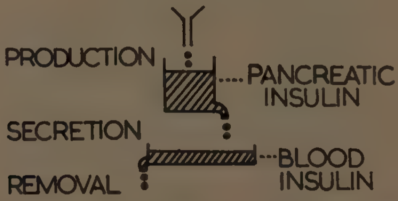

FIGURE 2. Diagram to show that the insulin level of the blood depends on the relation between the secretion of insulin into the blood and its removal (as by utilization, destruction, or excretion). The same reservations apply as for the insulin content of pancreas.

Studies on the isolated perfused pancreas also might permit a calculation of the total amount of insulin leaving the pancreas and hence of islet secretion, but in these experiments the conditions are even more abnormal. One could expect that factors involved in the production of insulin might be interfered with and that, in addition, the circulation to the islets would probably be affected in a way difficult to predict.

Other studies not directly involving insulin measurements may provide information relating to the secretion of insulin. These investigations involve the measurement of changes in certain materials or activities and the comparison of these with the changes produced by insulin. For example, when sugar is infused directly into the pancreatic artery in small amounts the general blood sugar level will fall, whereas this amount of sugar injected elsewhere may produce no change or, if anything, a rise in blood sugar level. This fact would seem to be good presumptive evidence for insulin secretion by the pancreas. However, if an agent thus introduced into the pancreatic artery failed to produce a fall in blood sugar level, this might be because it failed to stimulate insulin secretion, or because the secretion of something else (for example, glucagon) had obscured or altered the effect. Conceivably, also, there might be some action of the agent itself on the liver. Thus, there are limitations to the use of this method, especially when agents other than glucose are used to stimulate the islets. Also, it is difficult to make these observations quantitative in relation to insulin secretion.

Insulin injections will cause the glucose-6-phosphatase activity of the liver to be reduced. Changes in glucose-6-phosphatase can be used as corroborative evidence of insulin secretion if the experiment is of sufficiently long duration. However, these changes can help to confirm only what is suspected from other information.

Direct visualization of the islets in living animals has not been used extensively in studies on islet secretion. Many years ago O'Leary[2] described changes in the islets of living mice that he thought were concerned with secretion. Little has been done subsequently in this field, although it merits more attention.

Finally, changes in islet growth can give some indication of prolonged stimulation or reduction of islet activity. Such changes have been studied by noting alterations in the total islet mass when the animal as a whole has been treated, or by noting changes in the islets when the pancreas itself has been perfused. These changes require time to become manifest, and they seem to occur only when increased islet activity has been present over a sufficient period.

These various procedures have been used in studying the secretory function of the islets of Langerhans. When properly used, measurements of the insulin content of the pancreas, taken in conjunction with the histological changes, still provide as good evidence as can be obtained for changes in insulin secretion in a great variety of conditions. However, if one could add to this satisfactory observations on the blood insulin level, then the interpretation would be aided. Using the various procedures, it has been possible to gain some idea of factors influencing the activity of the islets. While evidence of the importance of certain regulating factors is good, yet the way in which they bring about secretion is not clear, nor is the final common factor, if there is one, known with certainty (FIGURE 3). One may say, however, that the fundamental final common factor in the regulation of insulin secretion is probably humoral. The evidence indicates that nervous regulation is not of fundamental importance, since the pancreas can regulate insulin secretion remarkably well even though denervated or transplanted. One humoral factor of importance must be glucose. It is conceivable that the level of glucose in the blood going to the pancreas is the fundamental stimulating factor insofar as immediate secretion is concerned. Another factor of importance must be the level of insulin in the blood. Insulin administration depresses islet function, but it is possible that the insulin effect is primarily on insulin production rather than secretion, since the insulin content of pancreas falls, presumably because, initially, the production of insulin is reduced to a greater extent than its secretion. The pituitary gland does not seem to be fundamental to the regulation of insulin production and secretion, since there is evidence that large changes in islet activity can occur in hypophysectomized animals. However, it is conceivable that the basic stimulating factor is glucose, and that other agents such as insulin, anterior pituitary substances, cortisone, and thyroxine, produce their effects by altering the ease of access of glucose to the cell or by influencing the sensitivity of the insulin-secreting mechanism to glucose. Whatever the fundamental final common path may be, many factors do influence islet activity and growth, and some of these still act in the absence of the pituitary gland. One further factor must

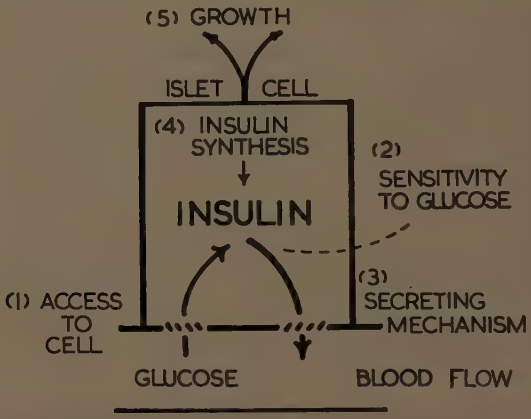

FIGURE 3. Diagram to indicate some possible ways in which the secretion of insulin might be influenced (1) by altering the access of glucose to the cell, (2) by altering the sensitivity to glucose of the insulin-secreting mechanism, (3) by changing the secreting mechanism itself, or (4) by altering insulin synthesis. In addition, secretion may be influenced by changes in the blood flow through the islets. Changes in secretion may in turn influence the total amount of islet tissue.

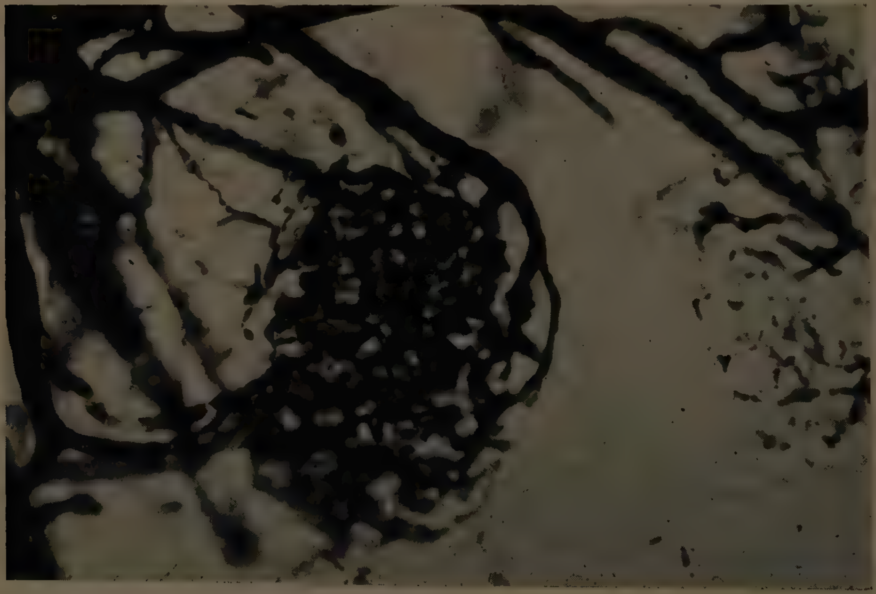

FIGURE 4. India ink-injected vessels of an islet, showing the rich blood supply.

be mentioned. The circulation to the islets must have an influence on secretion. The islets are highly vascular structures (FIGURE 4). Changes in the circulation to them could be of great importance in the control of secretion but, unfortunately, very little is yet known concerning the way this circulation is regulated and what relation it bears to the activity of the islets.

The cells of the islets seem to be able to vary the secretion of insulin within limits according to the need for it (FIGURE 5). Ordinarily, then, there may be a large variation in the secretion of insulin by the existing islet mass. In addition, the mass of islet tissue can be increased above the pre-existing levels. In the normal growing rat anything that greatly increases the insulin requirements over a period of time will lead to an increase in the mass of islet tissue. This increases the potentiality for insulin secretion and permits enhanced secretion while maintaining a reserve of function.

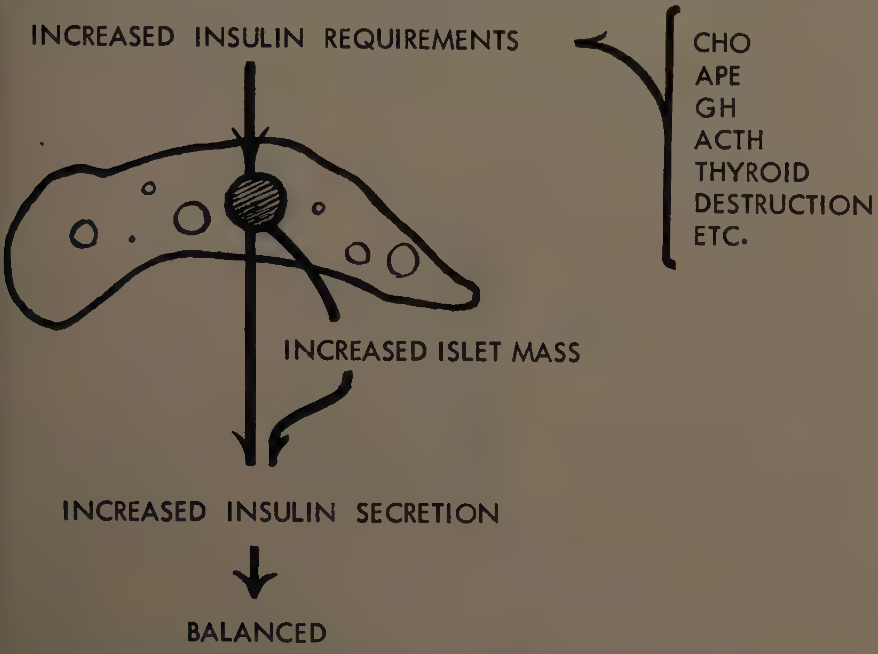

FIGURE 5. Diagram to indicate a number of factors increasing insulin requirements: CHO = carbohydrate, APE = crude saline extract of the anterior pituitary gland, GH = growth hormone, ACTH = adrenocorticotropic hormone, THYROID = desiccated thyroid, DESTRUCTION = enhanced insulin breakdown or removal. The increase in insulin requirements leads to insulin secretion and, if this is extensive and sustained, to an increase in the amount of islet tissue, thus increasing the potentiality for secretion.

It will not be possible to deal with all aspects of the regulation of insulin secretion at this time, but an attempt will be made to review briefly the evidence for some of the regulating factors. The factors influencing the islets may be divided in a general way into those that reduce islet activity and inhibit islet growth and those that increase islet activity and enhance islet growth. The term growth is used here, in an arbitrary way, simply to mean an increase in the total amount of islet tissue without specifying whether this is a consequence of an increase in the number of islet cells or of an increase in the size of individual cells or both and without indicating the cell types involved in the increase. There is evidence to support the view that islet activity can be reduced, and/or islet growth can be inhibited, by the injection of adequate amounts of insulin, by the restriction of caloric intake, by the restriction of carbohydrate intake and by removal of the pituitary gland, and that islet activity can be increased and islet growth enhanced by increasing the carbohydrate or carbohydrate-forming substances in the diet, by infusing sugar intravenously, by administering anterior pituitary extracts, growth hormone, or ACTH, by administering thyroid hormone, by injecting steroids of the adrenal glands or of the sex glands, or by administering certain sulfonylurea compounds.

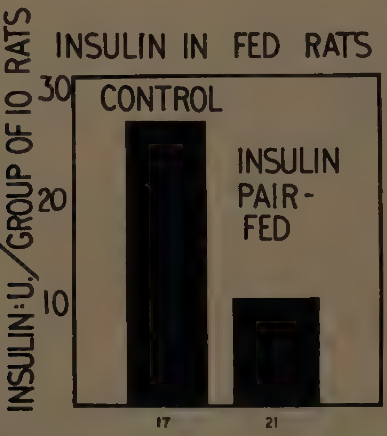

FIGURE 6. The effect on the insulin content of pancreas of adequate insulin administration in fed rats. The insulin-injected rats were pair-fed with the controls.[28]

Investigations concerning a number of different endocrine glands indicate that the injection into an animal of the hormone produced by a particular gland reduces the production of that hormone by the gland. When adequate amounts of insulin are given over a period of time to rats, several effects are noted. First, there is a profound reduction in the granulation of the beta cells of the islets. (Incidentally, this reduction in granulation is more difficult to demonstrate in the rabbit than in the rat.) The reduction of granulation of beta cells in the rat is associated with a reduction in the insulin content of pancreas. The injection of 1 to 3 U. protamine zinc insulin daily for 1 week caused a marked reduction in the insulin content of the pancreas (FIGURE 6). This reduction in insulin content cannot be ascribed to a corresponding reduction in islet mass but rather to a reduced concentration of insulin per unit of islet tissue. In growing rats it was not possible to demonstrate a reduction in the total amount of the islet tissue below the initial levels by the administration of insulin, but the normal increase in islet tissue was inhibited (FIGURE 7).[3] Insulin thus reduced the specific granulation of beta islet cells, reduced the concentration of insulin in islet cells, and inhibited the growth of the islets. These effects are considered to be due to reduced activity of the islets, a conclusion substantiated by the fact that when the insulin administration was stopped the animals temporarily became diabetic.

Restriction of the food intake also reduces the activity of the islets and islet growth. When rats were fasted for 1 week the insulin content of the pancreas was reduced to about one-half the normal value (FIGURE 8);[4] when insulin was injected into the fasting rats, the insulin content of pancreas was reduced to much lower values.[5] This observation would seem to indicate that the effects of fasting and of insulin administration are in the same direction and would support the conclusion that the reduction in the insulin content of the pancreas with starvation results from a reduced activity of the islets. With one week of fasting, there was no good evidence that the total amount of islet tissue was much reduced; hence, the reduction in the insulin content of pancreas involved a reduction in the concentration of insulin in the islet cells. However, when young rats were fed amounts of a diet just sufficient to maintain their body weight but insufficient to permit body growth, the islets also failed to grow (FIGURE 9).[6] It is concluded, then, that restriction of caloric intake reduces the insulin content of pancreas, inhibits islet growth, and reduces the activity of the islets, a conclusion that is supported by reports in the literature of starva-

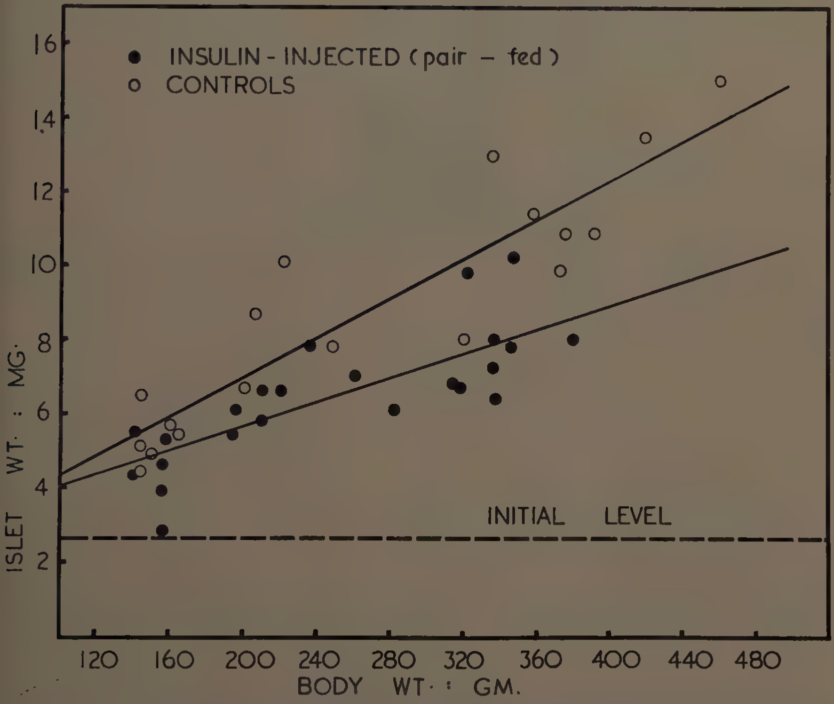

FIGURE 7. The effect of insulin administration on the growth of the islets of Langerhans. Reproduced with permission from the *American Journal of Physiology*.[3]

tion diabetes or "hunger" diabetes, a diabetes of transient type following a period of starvation or undernutrition.

There is a good deal of evidence to indicate that restriction of carbohydrate or carbohydrate-forming substances will produce an effect similar to that of

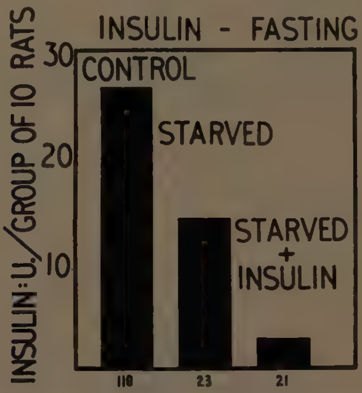

FIGURE 8. The effect of fasting and of insulin plus fasting on the insulin content of pancreas.[28]

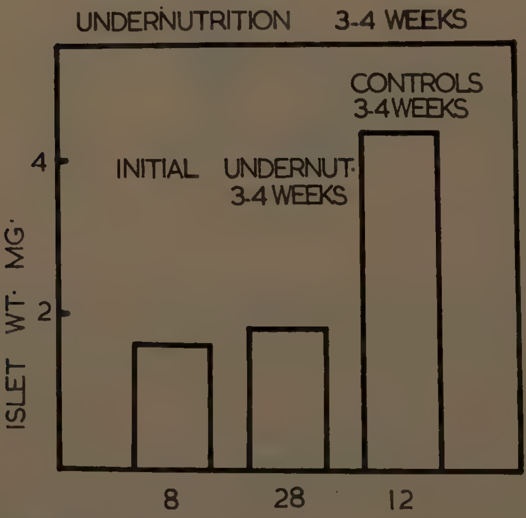

FIGURE 9. The effect on islet weight of keeping rats on a diet just sufficient to maintain their body weights (undernutrition). Control rats were fed ad libitum.

reduced caloric intake. When rats are fed a diet very high in fat there is a reduction in the insulin content of the pancreas that is as great as in complete starvation.[4] Here, too, the effect is enhanced by the simultaneous administration of insulin.[5] In experiments in which equicaloric amounts of fat and carbohydrate were fed, the animals fed fat showed a much lower insulin content of pancreas than those fed carbohydrate.

The effect of dietary constituents on the volume of islet tissue was demonstrated further by placing rats on a basal diet that was just sufficient to maintain body weight and then adding to this diet equicaloric supplements of either fat, protein, or carbohydrate (M. A. Ashworth and R. E. Haist, unpublished data). In 3 to 5 weeks the animals on the carbohydrate supplement and on the protein supplement showed a significant increase in islet volume, whereas those on the fat supplement did not. From these experiments it would appear that there must be sufficient amounts of carbohydrate or carbohydrate-forming substances in the diet if insulin production is to be maintained and islet growth is to continue.

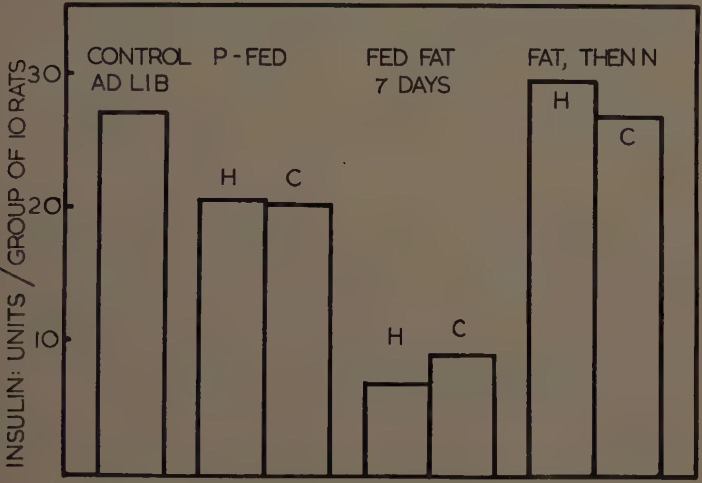

FIGURE 10. The effect of hypophysectomy on the insulin content of the pancreas. H = hypophysectomized rats; C = control rats; CONTROL P-FED = insulin content of pancreas of control rats pair-fed with the hypophysectomized rats; FED FAT 7 DAYS = insulin content of pancreas in hypophysectomized and control rats fed fat for 7 days; FAT, THEN N = insulin content of pancreas in hypophysectomized and control rats fed fat for 7 days and then restored to a balanced diet for 7 days.[28]

It is known from other work that the anterior pituitary gland exercises an important influence on carbohydrate metabolism and on many endocrine glands. Removal of the pituitary gland stops the growth of the rat, and the amount of food the animal eats is lessened. After a period of time following removal of the pituitary, the insulin content of the pancreas is diminished when compared to the values obtained in those rats receiving as much food as they would eat, but it is not reduced in relation to the levels in control animals receiving the same caloric intake (FIGURE 10). Moreover, if the hypophysectomized rats are fed a diet very rich in fat, the insulin content of the pancreas is reduced to much the same degree as in control animals, and the refeeding of a balanced diet following a period of 1 week on the fat diet brings back the insulin content of the pancreas to normal levels or even above them.[7] It would appear from this that, in growing rats, the removal of the pituitary gland does not prevent the changes in insulin production caused by feeding fat, that is, by restricting the carbohydrate intake; hence, the islets can alter their activity in relation to the need for insulin in the absence of the pituitary gland. In addition to this, it is found that removal of the pituitary gland does not cause a significant atrophy of the islets in young rats (FIGURE 11), although it does prevent further islet growth under ordinary circumstances.

It is possible to have a great reduction in the insulin content of pancreas without any marked decrease in the amount of islet tissue but, when one considers those factors that increase the insulin content of pancreas considerably above the normal values, it is found that the conditions increasing the insulin content of the pancreas in the rat also lead to an increase in the total amount of islet tissue. Whether or not this holds true for endocrine glands in general, it seems to be true, insofar as the endocrine pancreas is concerned, that whenever there is a sustained increase in the concentration of hormone in the gland an increase in the tissue producing the hormone has been found. A decrease in the hormone level in the gland, however, need not be associated with any measurable change in the total amount of hormone-producing tissue.

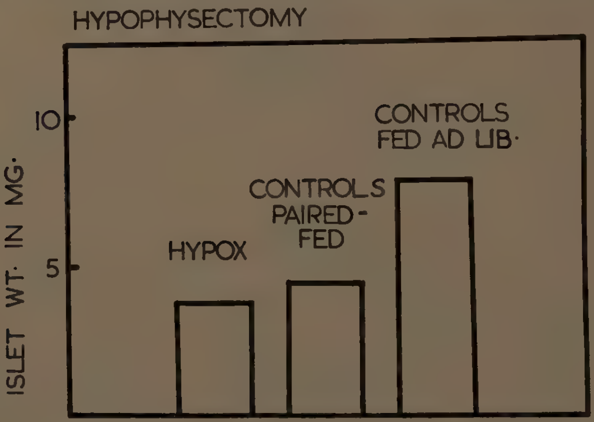

FIGURE 11. The effect of hypophysectomy (28 to 130 days) on islet weights of rats. The rats of one control group were paired-fed with the hypophysectomized rats and the rats of the other control group were fed ad libitum.

Daily injections of saline extracts of the anterior pituitary gland into rats have been reported to cause an increase in the insulin content of the pancreas[8] and an increase in the total amount of islet tissue.[9] The latter finding has been substantiated, and it has been shown in addition that certain highly purified anterior pituitary principles (growth hormone, adrenocorticotropic hormone (ACTH), and prolactin) have this effect also. Significant increases in islet tissue were found after the administration of each of these materials for periods of several weeks. The effect of growth hormone was obtained using subcutaneous injections for 4 weeks, but the best effect of ACTH was obtained when the material was given by continuous intravenous infusion for 1 week. FIGURE 12 shows the effect of growth hormone administration to hypophysectomized and intact rats.[10] The effect of ACTH is indicated in FIGURE 13. Within 1 week, as a result of ACTH infusion, the islet weight was almost doubled.[11]

Cortisone, one of the products of the adrenal gland, the target organ for ACTH, also caused a significant increase in islet volume. The effect of thyrotropic hormone was not tried, but the administration of desiccated thyroid gland for periods longer than 40 days led to significant increases in islet tissue.[12] One report indicated that the insulin content of the pancreas was increased.[13] The effect of the gonadotropic hormones was not tried, but the hormones of the target organs in the female (that is, the female sex hormones) and similar steroids did give significant increases in islet tissue. This held for the estro-

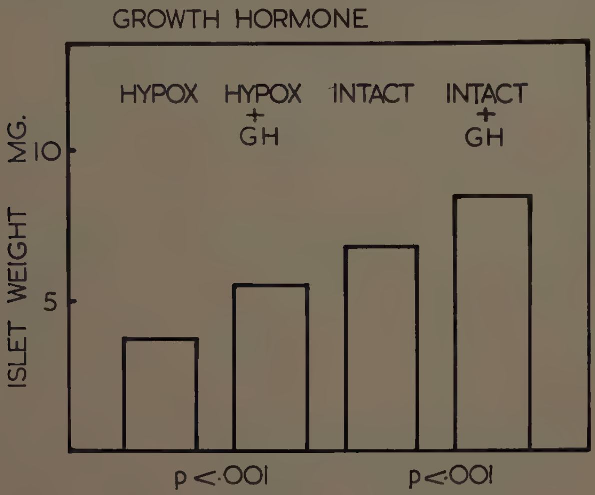

FIGURE 12. The effect on islet weights of growth hormone administration. HYPOX = hypophysectomized rats; GH = growth hormone-injected rats; INTACT = rats with pituitary glands intact.

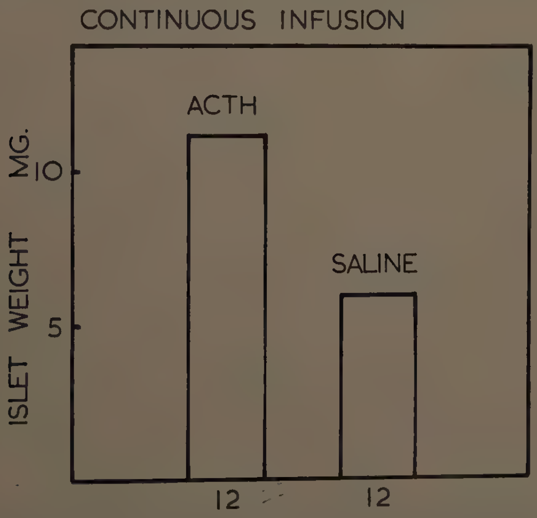

FIGURE 13. The effect of the continuous intravenous infusion of ACTH on islet weight in rats. ACTH = ACTH-infused rats; SALINE = saline-infused control rats.

gens, estradiol benzoate, diethylstilbestrol, and for progesterone, but administration of testosterone, the male sex hormone, did not produce any increase in islet tissue.[14] This observation is of interest also because of the report that the administration of certain estrogens increased the insulin content of the pancreas.[15] It seems evident from what has been outlined that the administration of a number of pituitary trophic principles or the hormones of their target organs leads to an increase in the total amount of islet tissue. There is always the possibility that this effect is mediated by one particular hormone, namely, a pancreatrophic pituitary hormone. However, it can be shown that several of these hormones or principles can produce their effects on the islets in the absence of the pituitary gland. Indeed, it is often possible to obtain a more pronounced effect in the hypophysectomized animal. Very good and significant increases in the total amount of islet tissue, in the absence of the pituitary gland, were obtained with growth hormone, ACTH, thyroid, and cortisone. The effect of the estrogens was not tried in the hypophysectomized animal.

It would appear, then, that a number of different, highly purified substances can stimulate the growth of the islets and increase the amount of islet tissue above the normal level. It is also evident that the stimulating effects of a number of these substances can be obtained in the absence of the pituitary gland; hence, it is unlikely that their effects are mediated by the pituitary through the release of a specific trophic hormone acting on the islets. It is felt that in the rat, under these conditions, the increase in insulin content of pancreas and the increase in the amount of islet tissue are in some way consequences of sustained increased insulin requirements and hence of increased islet activity. Studies of the insulin activity of blood[16-18] indicate that growth hormone administration for several days increased the insulin activity of blood in some species, but not in others. Investigations with pancreatic transplants in the dog[19] also gave evidence for increased insulin secretion following the administration of growth hormone.

One important effect on the islets is exerted by carbohydrate or carbohydrate-forming substances. Carbohydrate in the diet is essential for the maintenance of the insulin content of the pancreas and the growth of the islets. In addition, it can be demonstrated that, when large amounts of glucose are given by continuous intravenous infusion, the islet volume quickly and dramatically increases (FIGURE 14).[20] At the same time, there is some uneven degranulation of the beta cells of the islets during the course of the injections, but insulin estimations have not been done. This effect of a large glucose load on the islets is interesting because there is a good deal of evidence in the literature to indicate that the islets may be stimulated directly by increasing the glucose level in the blood going to the pancreas. In the isolated perfused pancreas it was found that increasing the glucose level of the perfusate led to the appearance of detectable amounts of insulin, indicating insulin secretion.[21] It was reported also that the cat given glucose intraperitoneally over a period of time exhibited degenerative islet changes and became permanently diabetic,[22] and it has been demonstrated that glucose given into the pancreatic artery led to a fall in blood sugar level and, after a period of time, to hyperplastic islet changes.[23] It has been indicated also that after glucose administration there may be as much as a tenfold increase in the plasma insulin level.[24]

When one follows the blood sugar level in rats injected continuously with glucose for a period of 7 days, it is found that the blood glucose level goes up initially but that, within about 24 hours, the level returns to the normal range unless the condition of the animals declines. (B. Kinash and R. E. Haist, unpublished data.) This can be seen in FIGURE 15. Despite the continuation of the infusion, the blood sugar level is not elevated for long. Nevertheless,

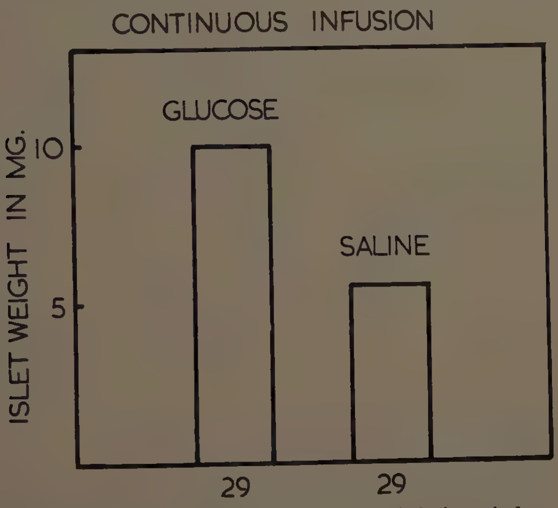

FIGURE 14. The effect of the continuous intravenous infusion of glucose on islet weight in rats. GLUCOSE = glucose-infused rats; SALINE = saline-infused rats.

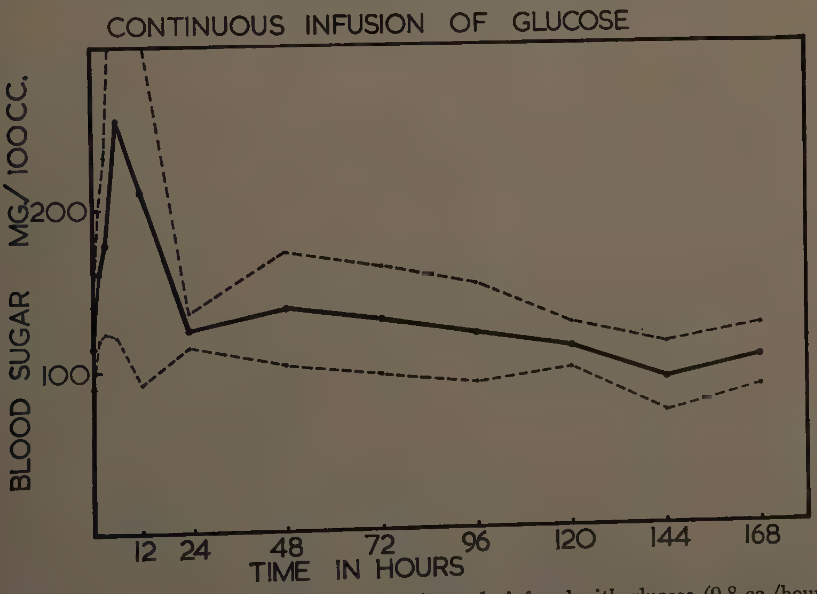

FIGURE 15. Blood sugar levels in rats continuously infused with glucose (0.8 cc./hour of 40 per cent glucose in 0.5 per cent saline). Mean values and standard deviations are shown.

since islet growth occurs, the islets are stimulated. This suggests that the level of blood sugar itself is not as important in this stimulation as the total amount of glucose given, or glucose load, or, possibly, access of glucose to the interior of the islet cell, or sensitivity of the insulin-secreting mechanism to glucose. Since this effect of glucose is such a good one, and since there is other evidence for a direct stimulation of the islets with glucose, it was of interest to discover whether the increase in islet weight could be obtained by the continuous infusion of glucose in the absence of the pituitary gland. When glucose was continuously infused into the hypophysectomized rat, it was found (Kinash and Haist, above) that a significant increase in islet tissue was not obtained, even though the amounts of glucose given were similar to those injected into the intact rats (FIGURE 16).

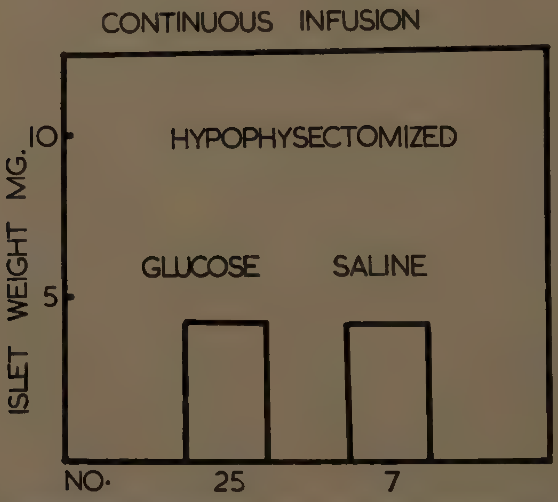

FIGURE 16. The effect on islet weight of the continuous intravenous infusion of glucose and saline into hypophysectomized rats.

The studies on the insulin content of pancreas would indicate that in the hypophysectomized rat there can still be regulation of the activity of existing islet cells in relation to changing insulin requirements. The basic regulation is still present. However, in the absence of the pituitary gland, islet growth is not stimulated by the glucose load. The blood sugar level does not rise as high initially in the hypophysectomized animals infused with glucose, but as shown in FIGURE 17, the sustained values are as high or a little higher than in the intact rats infused in the same way (Kinash and Haist, above). The loads and the levels of glucose were comparable, and yet the increase in islet tissue was not obtained when the pituitary gland was removed.

Because of the enhanced sensitivity of the hypophysectomized rat to insulin, the same glucose load may have provided actually a lesser increase in the insulin requirements. It would be important to know whether or not the direct stimulating effects of glucose injected into the pancreatic artery could still be obtained in the absence of the pituitary gland.

While under most circumstances those factors stimulating islet activity and growth also raise the level of blood sugar, in one experiment the islets appeared to be stimulated and to grow, even though the blood sugar was apparently not elevated and the insulin requirements apparently were not increased (FIGURE 18). This was when the sulfonylurea compound, BZ-55 (carbutamide) was given daily to rats for a period of 3 to 5 weeks.[25] The total amount of islet tissue in the pancreas was increased under these circumstances, and other evidence supports the conclusion that insulin secretion from the pancreas was stimulated by the administration of the drug. The blood sugar level of animals similarly treated was reduced, and the clinical evidence would lead one to conclude that the requirements for insulin would be reduced also. Hence, we cannot ascribe the stimulation of the islets or the increase in islet tissue obtained under these circumstances to an increase in blood sugar level or sugar load or to an increase in the requirements for insulin. This is possibly an instance of direct stimulation of islet activity by a drug, but we cannot be dogmatic about how the stimulating effect on the islets is obtained. In the rat the granulation of the beta islet cells is reduced as a result of the administration of carbutamide and, in the dog, the insulin content of the pancreas is greatly diminished. The insulin content of pancreas subsequently returns to normal levels despite the continuation of the treatment. This might result from an increase in the total amount of insulin-secreting tissue or merely from an increase in insulin synthesis in the existing islet cells that is sufficient to balance secretion.

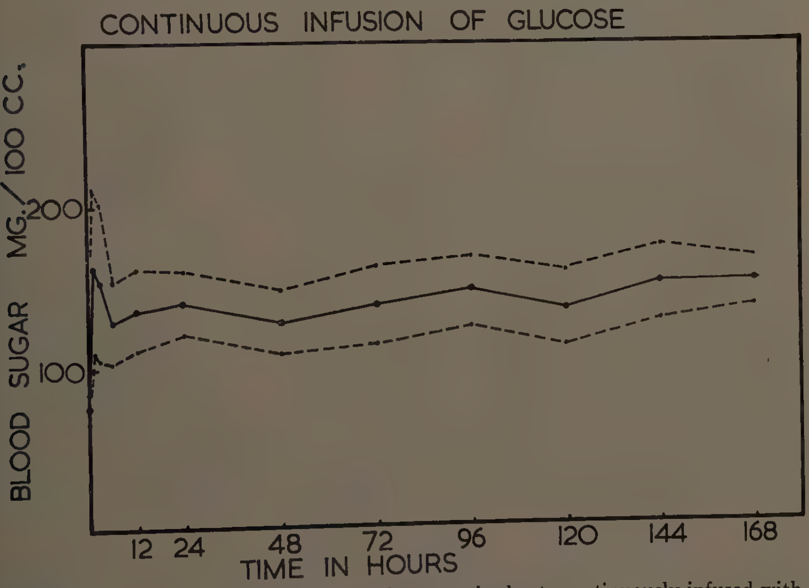

FIGURE 17. Blood sugar levels in hypophysectomized rats continuously infused with glucose. Mean values and standard deviations are shown.

It has been seen that certain factors reduce islet activity and islet growth. These are the administration of adequate amounts of insulin, restriction of food intake, restriction of carbohydrate intake, and removal of the pituitary gland. Other factors increase islet activity and enhance islet growth. These are administration of pituitary substances, or administration of hormones of the thyroid, adrenal, or female sex glands.

It is of interest to note that, when the amount of islet tissue is increased, the animal does not suffer from hyperinsulinism, that is, an excessive insulin secretion. In the normal animal, the secretion of insulin by the islet cells is regulated according to the requirement for insulin whether the amount of islet tissue be large or small. When hyperinsulinism occurs, therefore, it is an evidence of some abnormality in the mechanisms regulating insulin secretion rather than in the amount of islet tissue.

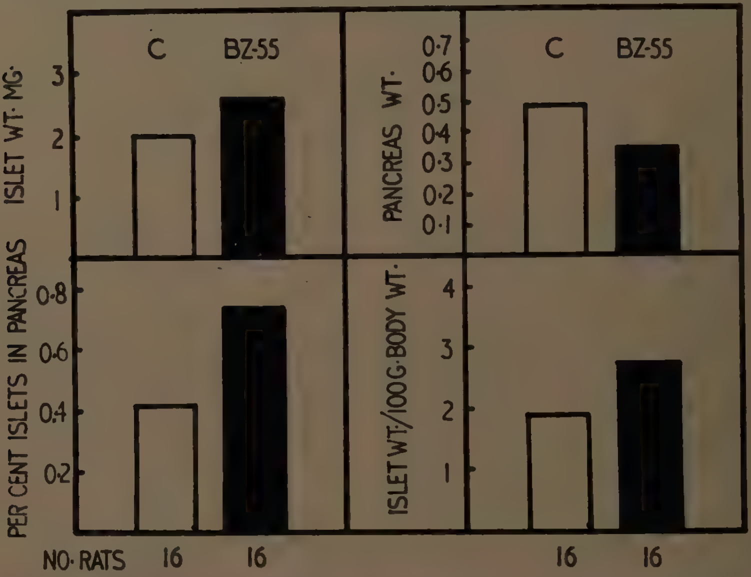

FIGURE 18. The effect of the administration of BZ-55 for 3 to 5 weeks on the islet weights and pancreas weights in rats. C = mean values for control rats BZ-55 = mean values for BZ-55-treated rats. Reproduced with permission from the *Canadian Medical Association Journal*.[25]

There is evidence that, under normal circumstances, insulin requirements are increased by a number of factors (FIGURE 5). If the requirement for insulin is increased, it seems likely that there will be a compensatory increase in insulin secretion by the islets. Although the evidence for this is mostly indirect, it is nevertheless good. If this increase continues, an increase in the total amount of insulin-secreting tissue (the islets of Langerhans) is obtained. Ordinarily, then, when there is an increase in the insulin requirement over a period of time, there is a compensatory increase in islet tissue. If there is a decrease in insulin requirement, as with excessive insulin administration, the growth of the islets is diminished. Thus, in growing rats at least, the islets of Langerhans are responsive structures important in homeostasis. It is felt that an important part of the response to a more sustained large increase in insulin requirements is an increase in islet tissue. In the normal individual an increase in insulin requirements will be balanced by an increase in insulin secretion and an increase in islet tissue. In the diabetic, this compensatory increase in insulin secretion and islet tissue, if it occurs, is not sufficient to balance the insulin requirements. Consequently, one way of thinking of diabetes is to consider it as, basically, a disorder in homeostasis or a disorder in compensation.

Not all species respond in the same effective manner as the rat to greatly increased insulin requirements. Most of the effects considered were obtained in growing rats, and one would expect that similar effects would be obtained in other species. This is true, but not necessarily to the same degree. Young growing dogs and cats probably are much like the rat, but in certain adult dogs and cats those factors that were shown to stimulate islet secretion and growth in the rat caused overstimulation, exhaustion, and degeneration of the islet cells. This can be illustrated by showing the effects of injections of anterior pituitary extracts. In the rat, these caused the islets to grow. With daily injections of anterior pituitary extracts in the dog, proliferation of islet cells was evident in some instances, hence growth was occurring, but was not sufficiently rapid and extensive to prevent the development of diabetes, and degeneration of the islets occurred (FIGURE 19).[26] If insulin was given along with the injections of anterior pituitary extracts, then the diabetes was prevented, as was the degeneration of the islets (FIGURE 20).[27] Similarly, the excessive stimulation and degeneration of the islets could be prevented by fat feeding or fasting during the period of APE injections.[28] The reason for mentioning these effects in

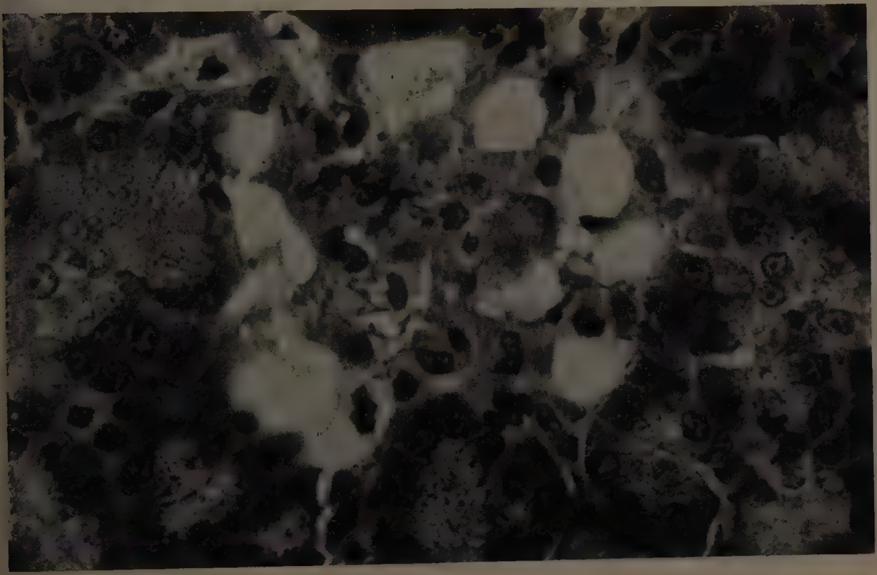

FIGURE 19. Photomicrograph of an islet from a dog that received 11 daily injections of an extract of the anterior pituitary gland. Reproduced with permission from the *American Journal of Pathology*.[26]

the adult dog is to point out the fact that while the basic responses in dog and rat are similar, namely, stimulation of islet secretion and growth, yet the end results are different. In the rat, growth is rapid and adequate, diabetes is not evident, and the increase in insulin requirements is balanced by increasing the insulin-secreting tissue and, hence, insulin secretion. In the adult dog, some growth of the islets does occur, but this is insufficient; the islet growth is not sufficiently rapid, so that the increased insulin requirements put excessive demands on the islet tissue and the cells degenerate. In the adult dog, then, the compensatory islet increase is not adequate, and diabetes results.

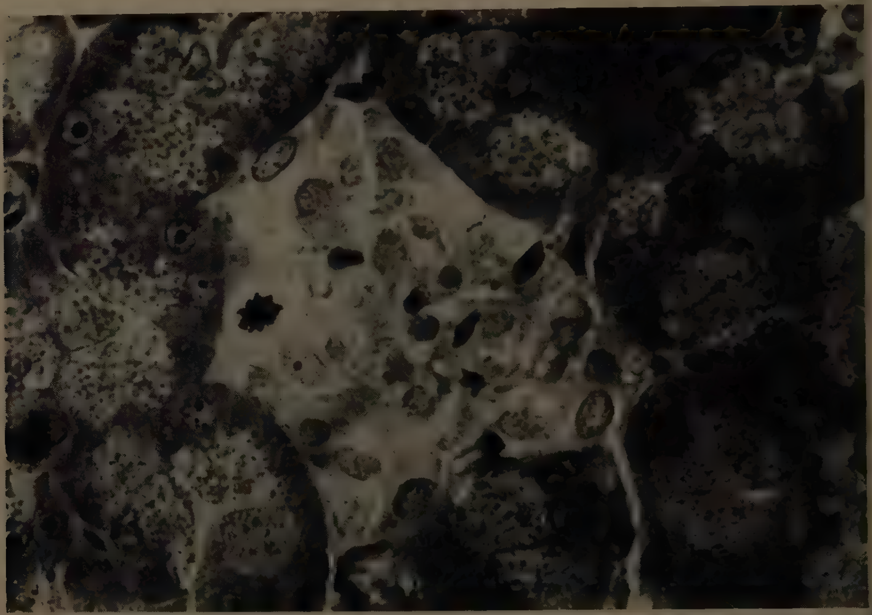

FIGURE 20. Photomicrograph of an islet from a dog receiving insulin in addition to injections of an extract of the anterior pituitary gland for 11 days. Reproduced with permission from the *Journal of Physiology*.[27]

Diabetes resulting from injections of anterior pituitary extract in the adult dog can be considered as a disorder of compensation. The absence of diabetes with similar treatment in the growing rat can be considered a result of adequate effective compensatory islet increase. The consequence of a continued large increase in insulin requirements in experimental animals is either an increase in the amount of islet tissue or degenerative changes in it.

How might the information obtained from the animals apply to man? In the animal or in man, diabetes is a condition in which there is a relative lack of insulin. The condition can be alleviated by injecting insulin. In the human diabetic whose diabetes begins in childhood, the condition is not unlike that of experimental diabetes in animals, there being destruction of the beta cells, a very low insulin content of the pancreas and, one presumes, an absolute lack of insulin. In certain diabetics whose diabetes has its onset somewhat later in life, there is still a considerable amount of apparently normal islet tissue, and the insulin content of the pancreas may be within the normal range. Since the individual is diabetic, there must be a relative lack of insulin. If the insulin lack in these diabetics were due to an increased utilization or destruction of insulin, that is, increased insulin removal, then this would greatly increase the insulin requirement. Since the requirements are not being met, there should be evidence of greatly increased sustained activity of the islets, namely, signs of either islet cell degeneration or islet growth. In some of these adult human diabetics there is little or no evidence of increased stimulation of the islets. If the demands for insulin in these patients are indeed excessive, nevertheless they are not met by normal compensatory responses. This would suggest that there is not a greatly increased requirement for insulin but, rather, that there is some interference with the secretion of insulin or the stimulation of this secretion.

However, islet atrophy is not extensive, and the insulin content of the pancreas is not greatly reduced in the pancreas of these patients. This would suggest further that the islets are still being stimulated, although this stimulation appears to be less than it should be at normal blood sugar levels.

The tentative conclusion is that in certain adult human diabetics at normal blood sugar levels the stimulation of insulin secretion by the islets is subnormal. Whether this is due to altered access of glucose to the islet cell or to altered sensitivity of the insulin-secreting mechanism to glucose or to some change in the secreting mechanism itself, or to an altered circulation to the islets is not known. It seems unlikely that it is due basically to a primary reduction in insulin synthesis, otherwise the insulin content of the pancreas should be low. While there is failure of islet growth to compensate for the insulin need, this would be a secondary effect consequent on the failure of stimulation of the islet cells.

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ELECTRON MICROSCOPY OF THE ISLETS OF LANGERHANS*

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Knowledge of the synthesis, storage, and liberation of insulin by beta cells is requisite to the attainment of the goals of either prevention or eventual cure of diabetes. Present knowledge indicates that direct stimulation of islets by high levels of blood sugar normally triggers the release of insulin. However, in many adult diabetics hyperglycemia seems to have lost this ability, although bioassays indicate that amounts of extractable insulin in the pancreas are adequate, and histological studies reveal that numbers of beta granules are frequently within the normal range.[1] Perhaps the interference here is not primarily with synthesis or storage of insulin, but with its ready liberation. Paradoxically, oral hypoglycemic agents administered to these cases often succeed where physiological stimuli have failed, because available knowledge indicates that at least one important action of these new drugs is to release some of the same insulin from beta cells that was not released by the stimulus of high levels of blood sugar. If this is true, then these drugs may be considered as keys that unlock stores of insulin previously inaccessible to the patient through the usual physiological mechanisms.

The problem of why the drugs can act when hyperglycemia cannot seems to be clearly formulated, and answers may finally rest with the biochemists and enzymologists rather than with the pathologists. However, if structural alterations in beta cells and islets share in the pathogenesis of diabetes in adults, morphologic studies, particularly at ultrastructural levels, may indicate pathways for the pursuance of solutions. We do not have answers yet, but the questions have stimulated my colleagues and me to attempt the elucidation of finer structures of beta cells with the electron microscope and to follow their changes under various physiological and pathological conditions. Abnormalities of islets in adult diabetics as recognized by light microscopy will be reviewed briefly, and results of recent studies of islet ultrastructure gained from experimental animals will be presented in the light of problems posed by studies in man.

Alterations in Islets of Adult Diabetics

As pathologists, we must state frankly at the outset of any description of islets in adult diabetics that, in an appreciable number (20 to 25 per cent), methods of examination used up to the present time have failed to detect abnormalities. Furthermore, those lesions that are demonstrable in the remainder of cases are not in any sense pathognomonic of the diabetic state because all may be found to certain degrees in the islets of some nondiabetics. Even when these changes are present, we cannot be sure always that they are significantly greater quantitatively in diabetics than in some nondiabetics of corresponding ages. Now that these shortcomings have been confessed openly, changes in the islets of diabetics that intrigue pathologists can be reviewed.

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* The work reported in this paper was supported in part by Grant A-1226 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

Degranulation with hydropic or glycogenic vacuolation (or both) is found at autopsy in only 5 to 15 per cent of adult diabetics. These cases, in which amounts of extractable insulin are significantly low, represent but a minority of the total, in contrast to a majority of juvenile diabetics, in whom islets are reduced in number or sometimes even completely absent,[2] and in whom the insulin content of the pancreas is low. However, in the remaining 60 to 75 per cent of cases of diabetes of age-onset type, abnormal deposits of fat (FIGURE 1) or hyalin (FIGURE 2) and collagen, as in fibrosis (FIGURE 3) constitute cardinal pathological changes. More than one of these abnormal materials (fat, hyalin, or collagen), and not infrequently all three of them, can be present within a single islet. Fat, in abnormal stainable form, and sometimes in large amounts, is probably the most ubiquitous. Lipid deposits almost always accompany hyalin, to such an extent that we prefer to refer to this combination as one complex: lipohyalin. Lipid may often be overlooked or forgotten because the usual methods of examination, such as paraffin sectioning, dissolve it from tissues.

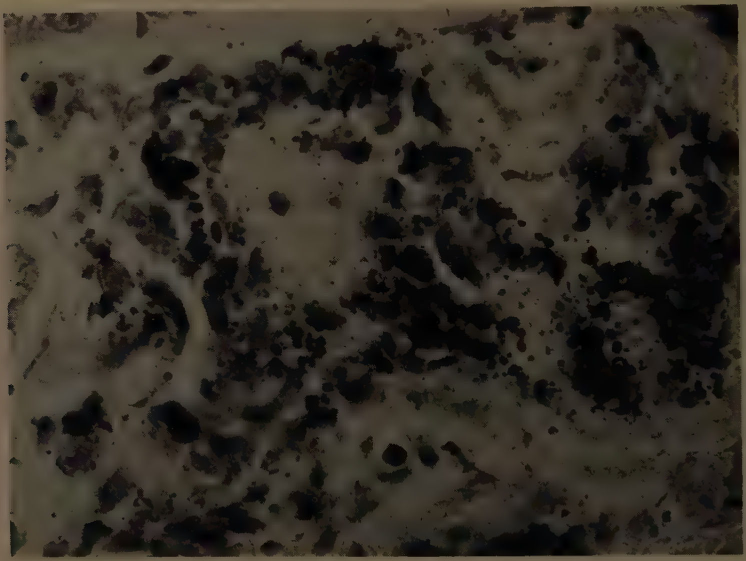

FIGURE 1. Lipohyalin is illustrated in this islet from an adult diabetic. Fat appears either black or gray, hyalin a lighter gray. Lipid droplets are present within the beta cells, obscuring their nuclei and rather scanty cytoplasm. The hyalin contains less intensely sudanophilic streaks of lipid. Frozen section stained with oil red 0 and hematoxylin. ×800.

Beta cells enmeshed within lipohyalin or collagenous bands along sinusoidal channels may appear to be relatively normal (FIGURE 4). They may be smaller than usual and their cytoplasm reduced. Sometimes their nuclei are abnormally small and darkly staining (FIGURE 3), but secretory granules may be as numerous as in beta cells of nondiabetics similarly examined. The temptation is strong to assume that abnormal deposits along or near basement membranes may act as barriers either to passage of glucose from blood to beta granule

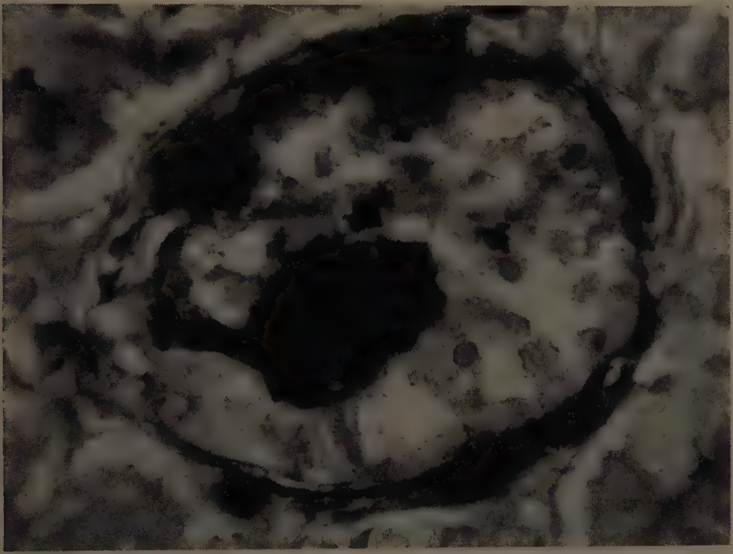

FIGURE 2. Hyalin appears black in this paraffin section of an islet from an adult diabetic (in frozen sections, lipid could also be demonstrated, as in FIGURE 1). Most of the hyalin is deposited in or around the capillary walls and appears to have narrowed the one in the center of the field almost to the point of occlusion. Some hyalin droplets can be seen intracellularly. Connective tissue stain. ×800.

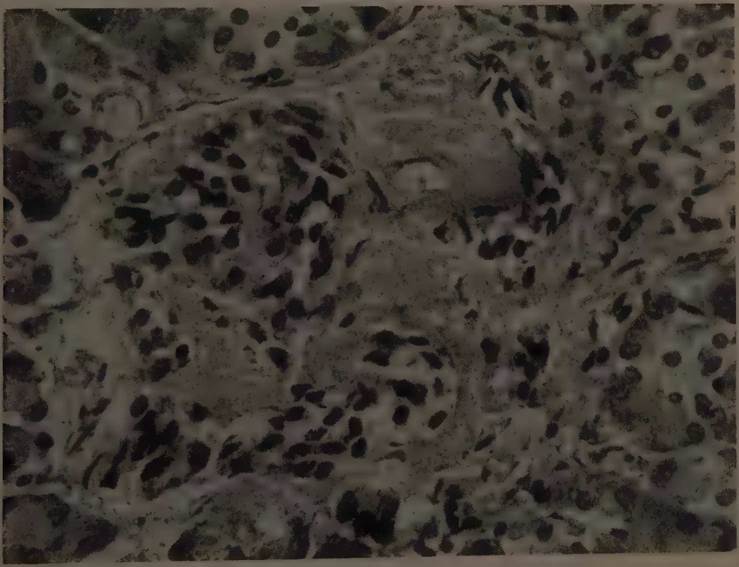

FIGURE 3. A mixture of hyalin and collagen is deposited in and around the sinusoids and beta cells of this islet from an adult diabetic. The collagen appears to be fibrillar, the hyalin smooth. The beta cells between have rather shrunken dark nuclei that may suggest some pyknotic change. Hematoxylin-eosin stain; paraffin section. ×800.

(to stimulate insulin release) or, conversely, to passage of insulin from beta cell to blood. If either hypothesis has any basis, how do oral hypoglycemic agents overcome these hindrances, if their effect should prove to be achieved in part by facilitating passage of insulin or blood constituents through abnormal lipohyalin membranes of islets? Electron microscopy makes possible the delineation of various membranes and spaces, both intracellular and extracellular, through which substances from the blood stream must pass to reach beta granules and through which the latter must pass to reach blood.

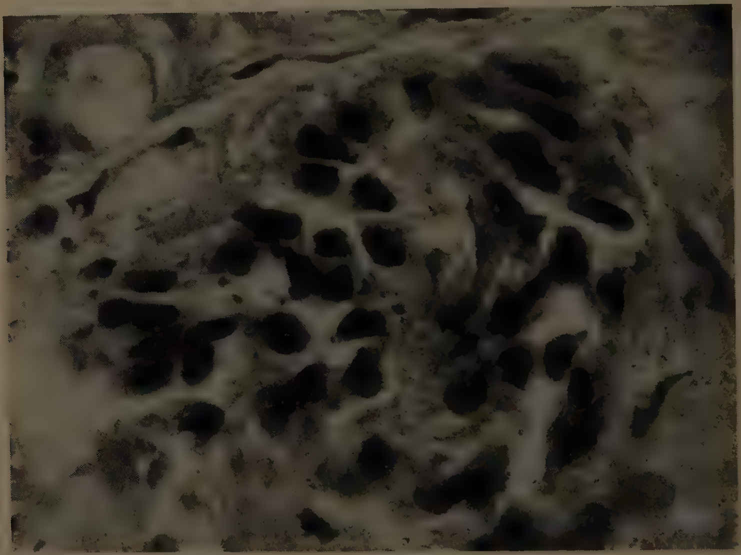

FIGURE 4. This nest of beta cells is surrounded by a meshwork of collagen and hyalin. The nuclei stain somewhat darker than normal, but the cytoplasm is not reduced, and granules can be seen. Gomori's chromium hematoxylin-phloxin stain. ×1000.

Structures Separating Blood from Beta Granules

Electron microscopic studies of islets from a number of species have enabled us to represent diagrammatically the spaces and membranes intervening between blood and beta granules (FIGURE 5). Before describing details in any particular species, a general concept based on all will be presented. The figures and abbreviations in brackets in the next paragraph refer to the diagram in FIGURE 5.

Leaving the capillary lumen (1), the first barrier encountered is the endothelial wall of the vessel, which consists of an inner plasmal membrane (2*a*), a thin component of ground substance (2*b*) in which may be found scattered small mitochondria, and the outer plasmal membrane (2*c*). At frequently recurring foci inner and outer membranes of endothelial cells are in almost direct contact, producing local attentuations of capillary walls (End. att'n.) The endothelium is separated by a small space (3) from the first of two basement membranes (4; BM_1), which appears more closely related to the blood vessel than to islet cells. This basement membrane (BM_1) is separated from a second (BM_2) by a wide space (5) in which processes of fibroblasts (Fib. Cyt. Pr.) are encountered. The second basement membrane (6) is closely related to islet parenchymal cells, from which it is separated by only a narrow space (7).

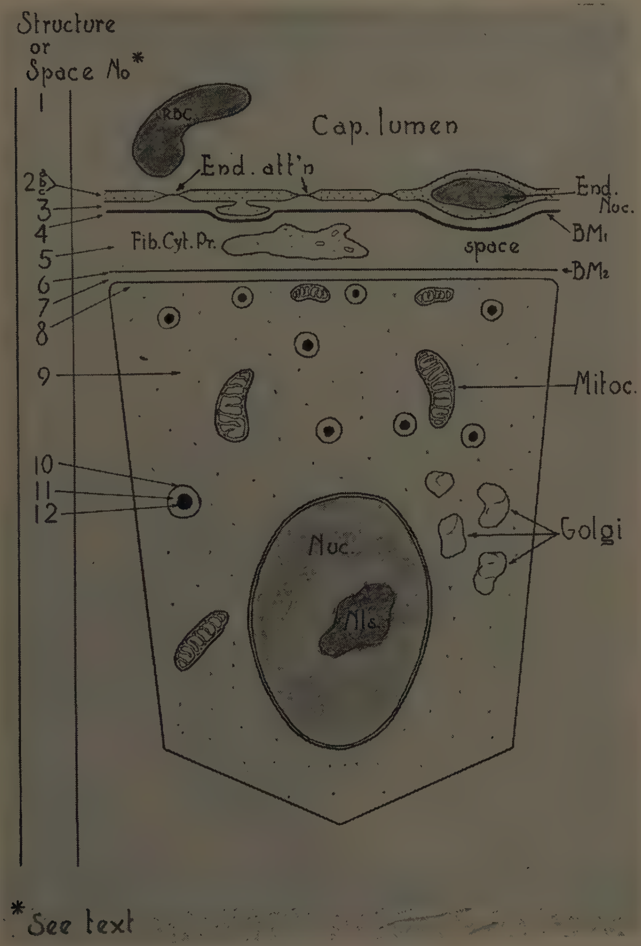

FIGURE 5. Diagrammatic representation of the ultrastructure of a beta cell, showing the relationships of its specific secretory granules (insulin or its precursor) to the lumen of the nearest capillary. The diagram represents a synthesis of studies of numerous islets in electronmicrographs prepared from a number of species. The figures on the left indicate the sequence of structures and spaces intervening between the blood stream and a granule, progressing in that order. See text for abbreviations and structure or space numbers.

Under certain circumstances described below, this space may become wider than it is normally. Neither basement membrane dips between adjacent beta cells, although the second one (BM_2) appears to be more closely related to islet cells than to capillary endothelium. To enter a beta cell, its plasmal membrane (8) must be penetrated to reach the cytoplasmic ground substance

(9) with its contained mitochondria (Mitoc.) and Golgi vacuoles (Golgi). Insulin in beta granules (12) is reached by traversing first the thin but definite wall (10) of the small saccule in which the granule lies and next by crossing the space (11) that separates the granule (12) from its saccule wall. At least 5 membranes and 5 spaces separate granules from blood. In theory, pathological alterations at any one or more of these 10 sites could interfere with insulin liberation.

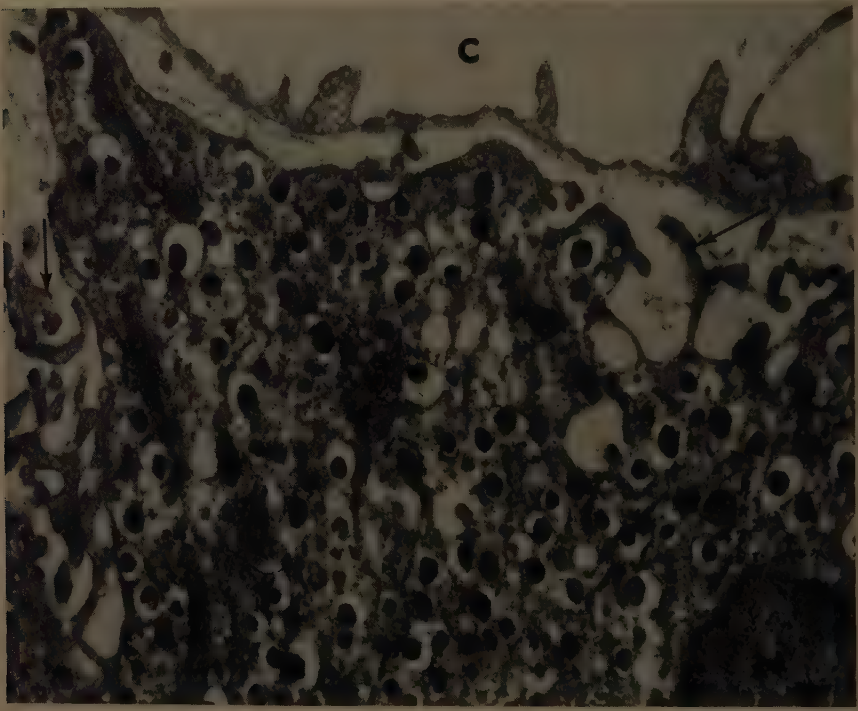

FIGURE 6. Electron micrograph of a beta cell of a rat after 2 days of treatment with glucagon and a high carbohydrate diet. Numerous microvilli (*arrows*) project into the intercellular and pericapillary spaces. The beta granules are round, homogeneous structures enclosed in membranous sacs. A distinct clear space is present around the granules. C = capillary lumen, N = nucleus. ×18,000.

Although evidence that insulin is stored in beta granules is strong,[1] some also is probably present in nongranular form. Because granules have not been observed in the process of extrusion from cells even under pathological conditions, it is quite likely that insulin may enter into nongranular form at some point either before or at the time it leaves the cell. Granules are too large to represent insulin in its simple molecular form, and may be complexes of molecules perhaps linked to other proteins to form "granule packages." Intriguing variations in form and size of beta granules in several species will be described. The mechanisms by which insulin escapes from its package are unknown. Perhaps the granule, still in its saccule, moves to the sinusoidal pole of the cell before leaving it but, after pathological stimulation in some species, empty granule saccules were found deep within beta cells. The latter observation suggests that insulin could leave its saccule at any site within the cytoplasm and traverse the ground substance toward the capillary in some soluble, non-

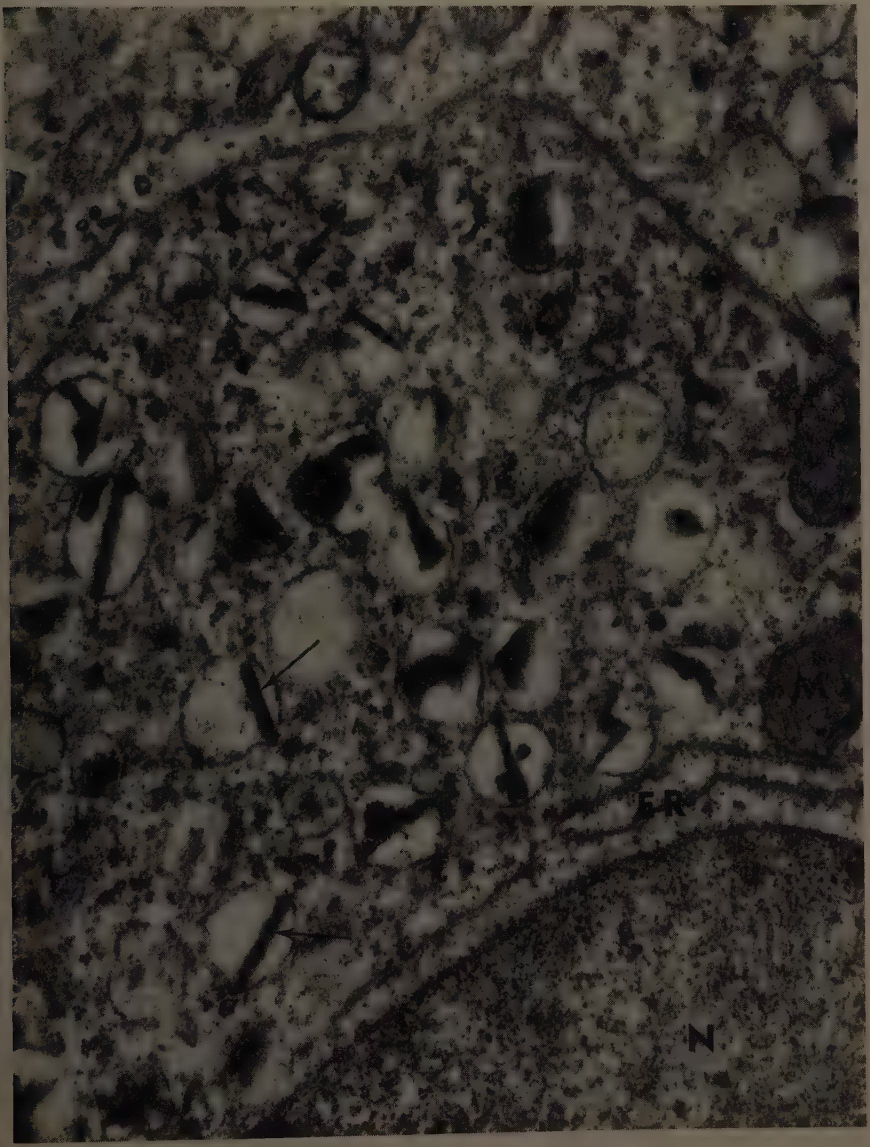

FIGURE 7. Electron micrograph of a normal beta cell from a dog prior to the injection of Orinase. Many of the granules (*arrows*) have rectangular profiles and are enclosed in membranous sacs. Ergastoplasm (ER) and mitochondria (M) are scattered diffusely throughout the cytoplasm. N = nucleus. ×24,000.

granular form. Granules have not been observed free of their enveloping saccules.

By whatever mechanisms insulin reaches sinusoidal borders of beta cells, the membranes here in most species are irregularly thrown into a few, fine projecting processes or microvilli. Granules have not been seen within villi, which suggests that at least somewhere in this vicinity, insulin must exist in nongranular solution. These villi have been observed to increase in number following the application of certain stimuli,[3] as in glucagon administration in the rat (FIGURE 6). Perhaps under some conditions numbers and size of villi may be an index of rate of liberation of insulin from beta cells. Villi could result from the fusion of the wall of a granule saccule with the plasmal membrane. The focal rupture of both at the point of fusion on the surface would leave projecting fragmented ends, resulting in the appearance of two villi on either side of a small concavity. Such a process could be termed reverse pinocytosis or, to introduce a single word for such an event emiocytosis (ejection from a cell).

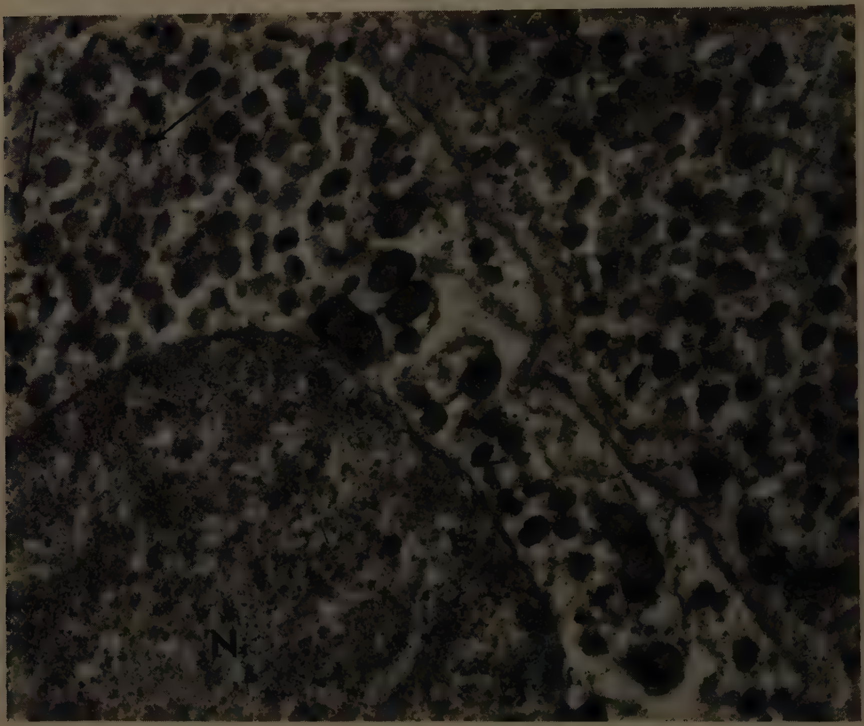

FIGURE 8. Electron micrograph of portions of normal beta cells of the cat. The granules have a central dense structure embedded in a less dense homogeneous substance. The dense structure has a rhomboidal shape in some granules and a circular or elliptical shape in others. N = nucleus. ×24,000.

From the foregoing it is regrettably clear that electron microscopy has failed to establish how insulin escapes from beta granules on through cell membranes to extracellular positions. We are equally at a loss to follow its movement on into sinusoidal lumens. In theory, abnormal intracellular or extracellular deposits of fat, hyalin, or collagen, either on membranes or in any of several intervening spaces, could lower normal degrees of freedom of passage of glucose from blood to beta granule or of insulin from granule to capillary lumen. We have no evidence that this type of hindrance is ever a real pathogenic factor in diabetes, and answers must await electron microscopy of islets in man, a program on which one of us (P.E.L.) is embarking with the co-operation of our surgeons. Methods have not yet been discovered for producing in animals a diabetic state that is coexistent with normal amounts of extractable pancreatic insulin and normal complements of beta granules with or without fatty, hyaline, or fibrotic change as encountered in adult diabetic man. Without this experimental model, which is so sorely needed, we must depend upon biopsy material obtained from man.

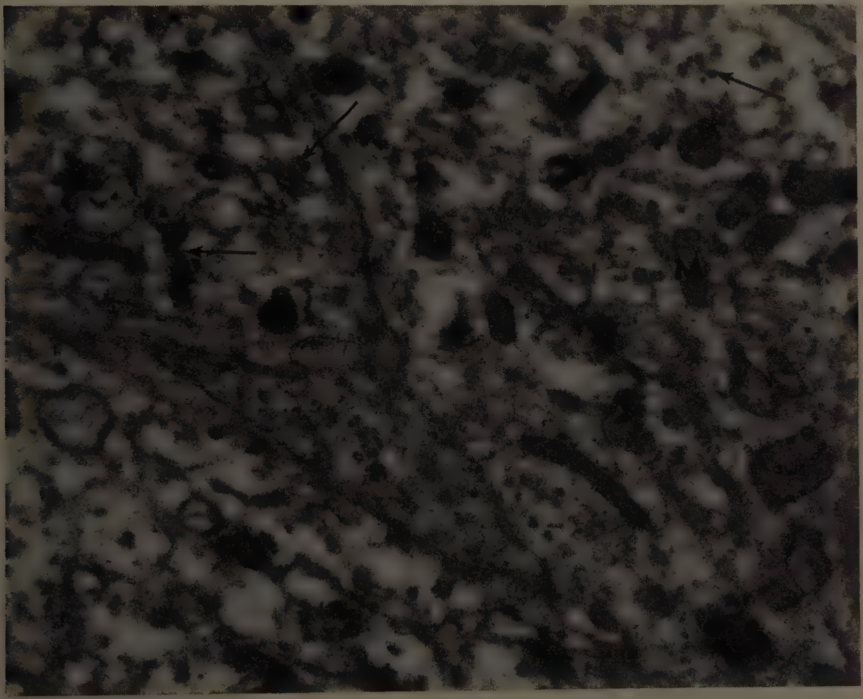

FIGURE 9. Electron micrograph of a portion of normal beta cells of the chicken. The granules have a definite crystalline structure. They are composed of sheaves of needle crystals that appear as small dots in cross section and as longitudinal strands when they are cut tangentially (*arrows*). M = mitochondria. ×24,000.

Variations in Form and Number of Beta Granules with Species and under Abnormal Conditions

In the foregoing, two pathological changes in islets have been considered as possible factors in the pathogenesis of diabetes in man: (1) reduction in insular tissue or (2) interposition of an abnormal barrier between beta granules and blood. A third possibility is a pathological change in the structure of granules in some diabetics. The similar appearance by light microscopy of beta granules in normal and diabetic man and in various animal species does not constitute a valid objection to this theory. The objection is untenable because, in contrast to their uniform appearance by conventional microscopic techniques, electron microscopy unexpectedly revealed distinctive and unique variations in the ultrastructure of granules in each species examined to date.[4, 5] These differences were completely unanticipated before the advent of the electron microscope. Granules of alpha cells do not share in this intriguing discovery because they are relatively uniform in shape, varying from animal to animal only in the size of their consistently circular profiles.

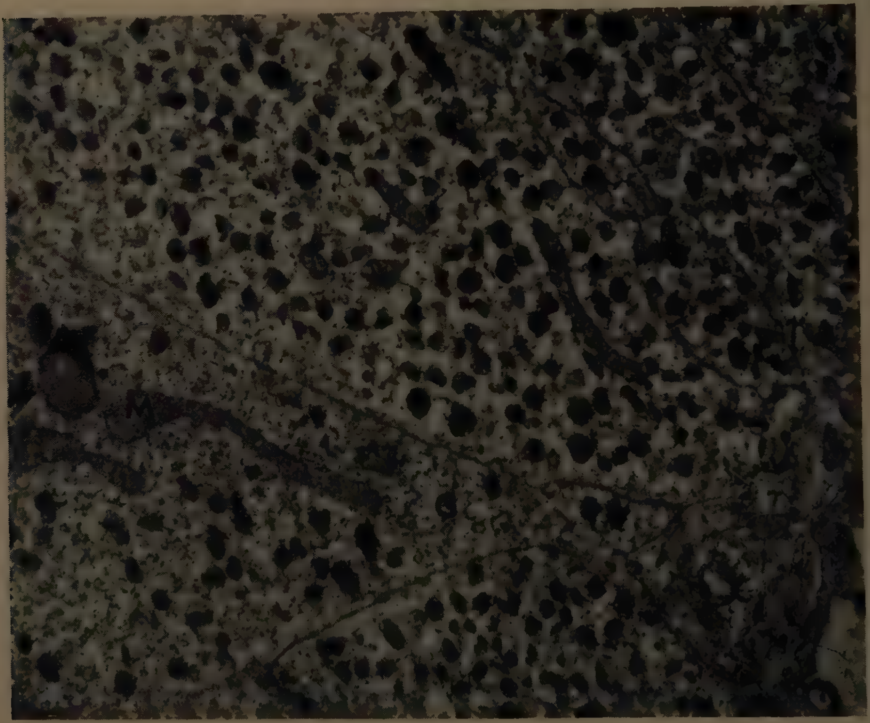

FIGURE 10. Electron micrograph of portions of beta cells from a cat that had received repeated intraperitoneal injections of glucose. Glycogen (G) is scattered diffusely throughout the cytoplasm and appears as light gray branching chains. Beta granules are still evident in these cells. M = mitochondria. ×18,000.

Beta granules of the rat (FIGURE 6) are round and homogeneous, but in dogs (FIGURE 7) they have a rectangular profile. In cats (FIGURE 8) they have a central, dense, rhomboidal body embedded in a less dense homogeneous substance. In chickens (FIGURE 9) the granules are distinctly crystalline and are composed of sheaves of needles. These examples of ultrastructural variations indicate that endogeneous insulin, or an intimately associated binding protein within the granule complex for each animal, is distinctly and characteristically species-specific. Studies are in progress in this laboratory to discover the ultrastructure of beta granules in normal man and to investigate the possibility of abnormal forms existing in beta cells of diabetics.

Changes in granule content and the abnormal accumulation of glycogen that develop in beta cells of cats subjected to hyperglycemia following repeated intraperitoneal infusions of glucose solutions have been studied by electron microscopy. Poles of cells adjacent to capillaries contained many granules, whereas opposite ones were filled with a lamellar type of ergastoplasm. Abnormal deposits of glycogen were demonstrable by light microscopy in islets of injected animals. Two patterns of deposition were evident by electron microscopy; in one, glycogen formed a meshwork of gray, branching chains scattered diffusely throughout the cytoplasm (FIGURE 10). Beta granules, although present, were fewer than in the controls. The second pattern consisted of focal accumulations of glycogen that displaced surrounding organelles (FIGURE 11). Abnormal collections of fluid were apparently associated with some of these deposits, because the delicately branching strands of glycogen

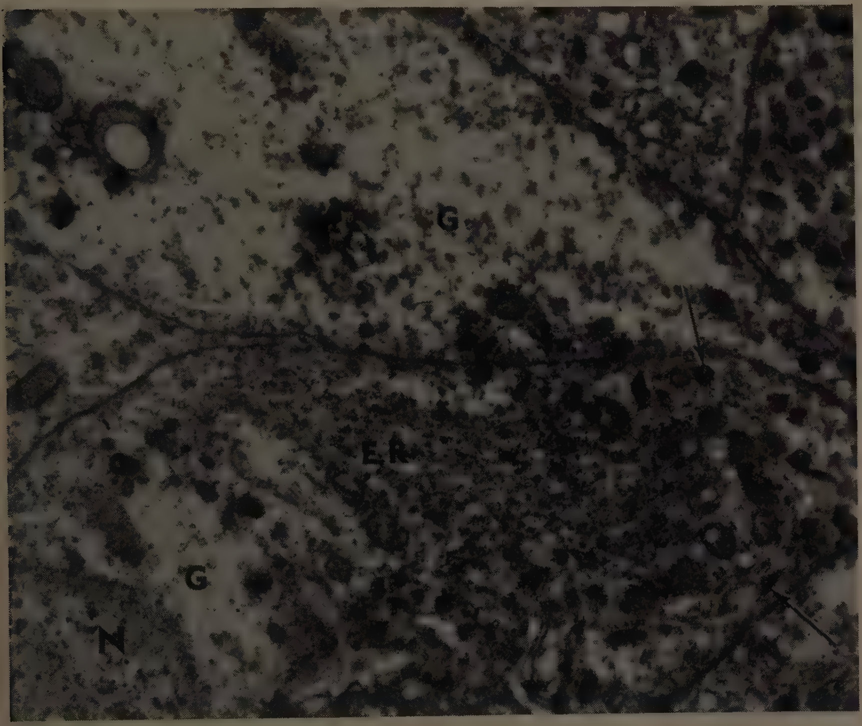

FIGURE 11. Focal accumulations of glycogen (G) are illustrated in portions of beta cells of a cat following repeated intraperitoneal injections of glucose. The branching strands of glycogen are separated by clear spaces of fluid. The beta granules (*arrows*) are decreased in number. The ergastoplasm (ER) is abundant in some cells and has a lamellar structure. N = nucleus. ×18,000.

were separated widely by clear areas. This appearance indicates that hydropic change had developed in addition to glycogenic accumulation per se. It is a distinctly different type of hydropic degeneration from that induced in alpha cells by cobaltous chloride[6] because, with the latter, the vacuoles were limited by ergastoplasmic membranes; membranes of any type were absent around hydropic spaces in feline beta cells that contained glycogen and fluid (FIGURES 11 AND 12).

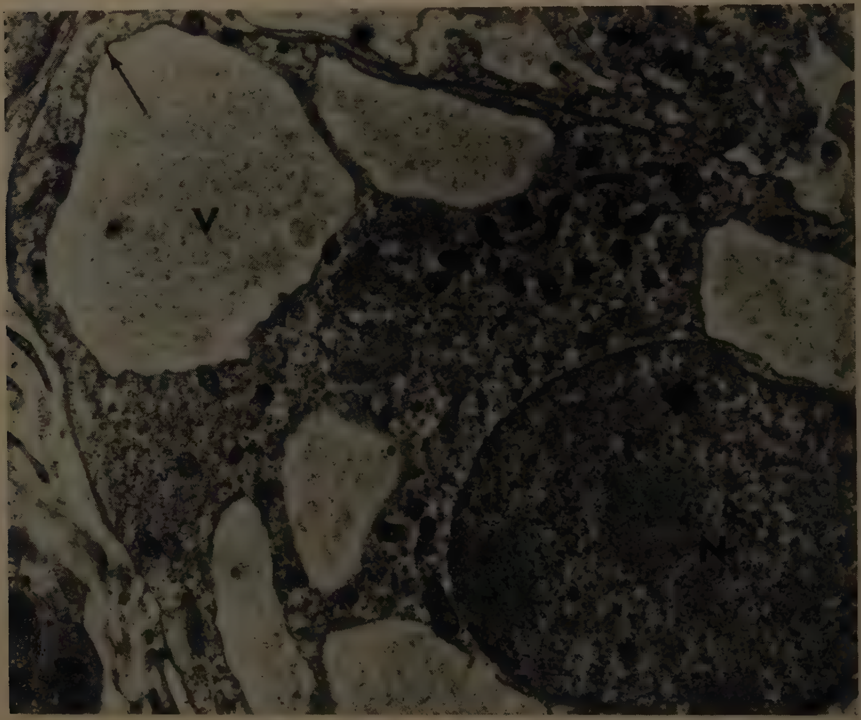

FIGURE 12. Electron micrograph of a hydropic alpha cell of a guinea pig after 13 days of treatment with cobaltous chloride. The vacuoles (V) are limited by a distinct membrane (*arrow*). Mitochondria and ergastoplasm are present between the vacuoles. A few alpha granules were present in other portions of the cell. N = nucleus. ×9000.

Acute experiments on dogs are in progress to study the effects on beta cells of intravenous administration of relatively large doses of tolbutamide (Orinase).* Initial findings indicate that under these conditions Orinase probably causes beta degranulation. Tissues of additional treated animals and the compilation of numerical indices of population densities of beta granules are being obtained before definite conclusions are drawn. The changes produced in these preliminary studies are illustrated in FIGURES 7 and 13. Numerous rectangular granules are present in the control biopsy (FIGURE 7), but only a few are present 2 hours after an intravenous injection of Orinase (FIGURE 13). Membranous

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* The Upjohn Company.

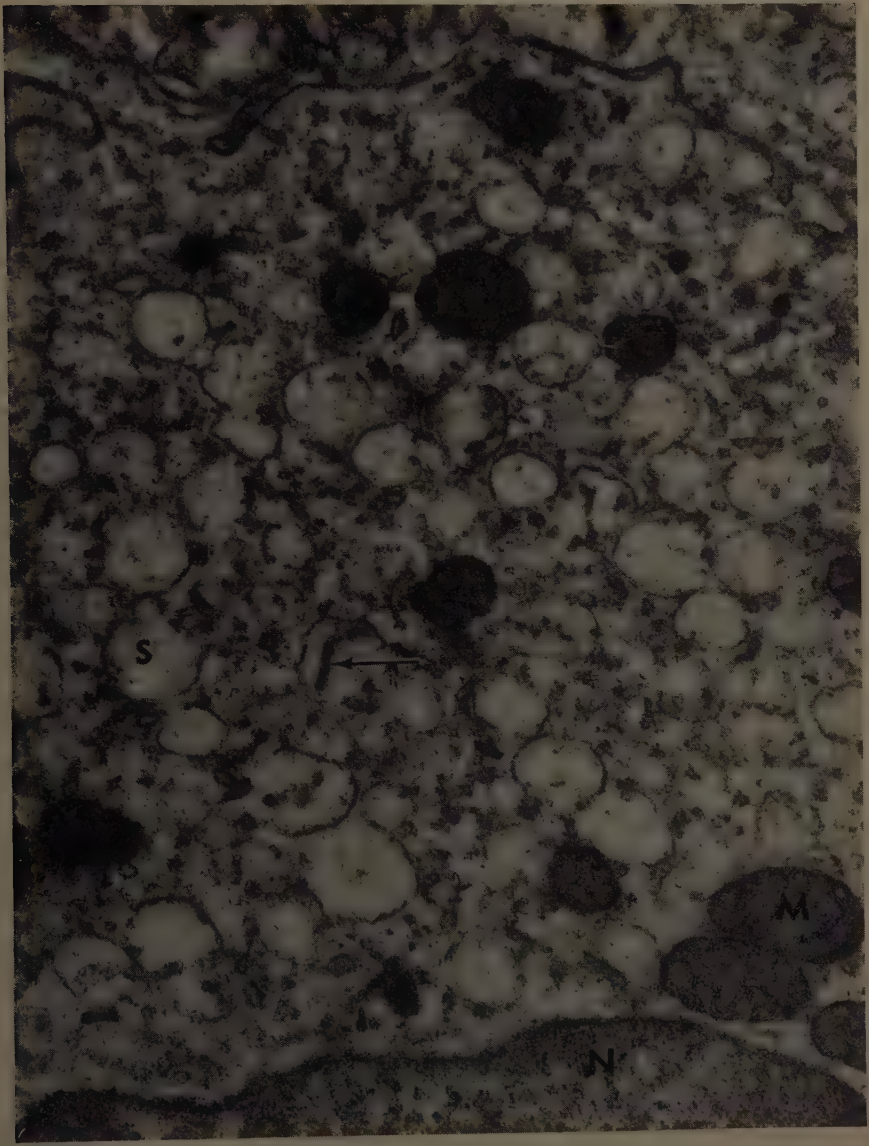

FIGURE 13. Initial observations on the ultrastructure of the beta cells of a dog two hours after an intravenous injection of Orinase. Degranulation of the beta cell is evident in the electron micrograph. Only one distinct rectangular beta granule (*arrow*) is present. The remainder of the membranous sacs (S) contain a small amount of amorphous material, but no distinct granules. These initial findings suggest that Orinase produces degranulation of beta cells; however, additional experiments and numerical indices of population density of granules are needed before a definitive statement can be made. M = mitochondria. N = nucleus. ×24,000.

granule saccules in these beta cells are empty but for small amounts of amorphous material.

Summary

Frequently encountered pathological changes in islets of adult diabetics include hydropic or glycogenic vacuolation of beta cells, abnormal deposition of lipohyalin on or around the vessel walls, fibrosis and, more rarely, decreases in numbers of islets. In 20 to 25 per cent of adult diabetics, islet abnormalities have not been demonstrated by techniques applied to man thus far. In all animal species studied to date, electron microscopy has shown that beta granules are separated from blood stream by a minimum of 5 spaces and 5 membranes, including walls of saccules in which they lie, plasmal membranes of cells, 2 basement membranes, capillary endothelium, and other structures. In theory, any of these spaces and membranes could be altered by the pathological deposition of either intracellular or extracellular fat, hyalin and collagen (singly or together), thereby threatening the normal degrees of freedom of interchange of glucose from blood to beta granule or insulin from granule to capillary lumen.

Diabetes has not yet been successfully produced in animals characterized by these kinds of abnormal insular deposits and by normal amounts of pancreatic insulin, which are features often present in diabetic man. Therefore, electron microscopic study of this type of diabetes must depend upon the availability of biopsy material obtained from patients at laparotomy.

The ultrastructure of beta granules is uniquely species-specific for all animals that have been studied. These species differences may represent either variations in endogenous insulin or differences in any binding proteins that, along with molecular insulin, may form granule packages or complexes.

Abnormal deposits of glycogen appear either as diffuse or focal delicately branching strands within the beta cells of cats injected intraperitoneally with large amounts of glucose solutions.

Preliminary studies of acute effects of intravenously administered Orinase on ultrastructure of beta cells in dogs suggest that degranulation occurs. Evaluation and interpretation of these findings await completion of additional experiments and collation of numerical incidences of population densities of beta granules.

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Discussion of the Paper

G. A. Wrenshall (*Toronto, Ont., Canada*): Lacy and Hartroft have stated that they are tempted to consider the increase in membranous structures that they see around and within the islets of Langerhans of many maturity-onset diabetic subjects as an effective barrier to the release of insulin. While such a barrier might account for the existence of diabetes in the presence of the fairly abundant beta cells frequently found in maturity-onset diabetic subjects, it appears to me to be incompatible with the effectively normal levels of plasma insulin that have been reported in such subjects.[1-3] How would Lacy and Hartroft interpret these findings in terms of their islet barrier hypothesis?

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P. E. Lacy and W. S. Hartroft: If membranous changes in beta cells or islets interfere with the normal release of insulin from beta cells to blood, the effect is probably not an all-or-none phenomenon, according to our hypothesis. Hyperglycemia or tolbutamide might stimulate the release of normal amounts of insulin into the blood of a maturity-onset diabetic subject by increasing the formation and concentration of insulin in the beta cells. Normoglycemic levels would be insufficient to accomplish the same degree of release in the diabetic subject. However, in a diabetic, hyperglycemia (if, paradoxically, it could exist) would therefore stimulate the release of greater than normal amounts of insulin, that is, sufficient insulin to meet the demands of greater than normal amounts of glucose in the blood. This theory would permit the existence of normal numbers of beta granules with normal levels of blood insulin and coexistent hyperglycemia (diabetes). Perhaps oral antidiabetic agents act to relieve the need for hyperglycemia to accomplish in the diabetic what normoglycemia in the nondiabetic does in terms of stimulation of release of insulin from beta cells. These considerations are, of course, hypothetical, since we have no experimental condition that reproduces this type of diabetes in animals; neither have we, as yet, electron microscopic studies on diabetic subjects with a normal degree of beta granulation.

USE OF THE GOLGI APPARATUS AS AN INDICATOR OF THE LEVEL OF ACTIVITY OF THE CELLS OF THE ISLETS OF LANGERHANS*

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The development of relatively sensitive methods for measuring insulin and glucagon in the circulating blood has been most valuable in estimating the level of islet function. However, all of these methods leave much to be desired in precision; moreover, there are circumstances in which their application is not easy. The alternative approach is to attempt to estimate the level of activity by histological means. Conventionally, the activity has been judged on the basis of the amount of specific granular material in the cells, the size of the cells, and changes in the size and appearance of the nuclei. The granule content may be very deceptive, however, since it obviously represents a balance between synthesis and release. It is possible, as will be shown, to show very definite depletion of granules not only in conditions of vigorous activity but also in conditions of extreme inactivity; moreover, the granule content may be unchanged under certain circumstances when other criteria indicate considerable reduction in activity. It obviously would be desirable to have another, more reliable, means for determining the activity. A most likely candidate for this role is the Golgi apparatus, for it has been shown repeatedly that in a large variety of secreting tissues, both exocrin and endocrine, the Golgi apparatus becomes very elaborate during high levels of secretory activity and, on the other hand, is much reduced in size when this activity is low. Widespread investigation of the exact nature and morphology of the Golgi apparatus in the living state is still in progress, and it appears quite likely that the appearance of this apparatus after impregnation with osmic acid is different from its appearance in life. However, for the type of study reported here, this question is relatively unimportant, for it is necessary only to show that this network, regardless of its precise nature, responds in a reproducible way in a variety of functional states. It can be shown that, in the islets, the Golgi apparatus responds quite rapidly and definitely to known stimuli, permitting exploration of function in a number of other conditions.

METHODS

Rats of the Long-Evans strain were employed exclusively. During all experiments the animals were kept on a routine stock diet ad libitum unless otherwise specified. In some experiments alloxan-diabetic rats were employed. Diabetes was induced by the intravenous administration of 50 mg. of recrystallized alloxan per kilogram of body weight as a 5 per cent solution in saline following a 72-hour fast.

In certain experiments glucose or insulin or both were infused intravenously through a catheter placed either in a tail vein or in a branch of the external

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* The work reported in this paper was supported in part by Grant No. A-1410 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

jugular. Nembutal anesthesia was used. Blood samples were withdrawn at intervals from the inferior vena cava for the measurement of the blood glucose by the Nelson modification of the Somogyi method.[1]

Following fixation in Bouin's fluid, specific granules were stained in 4-μ sections by the Gomori aldehyde-fuchsin method,[2] employing a chromotrope 2R-light green counterstain.

For demonstration of the Golgi apparatus, tissues were fixed in Champy-Kull fixative for 24 hours, washed for 24 hours, and treated with 2 per cent osmic acid for 8 hours at 56°C. and for 10 days at 35°C. They were then washed for 24 hours, dehydrated, embedded in paraffin, and sectioned at 4 μ. Excess deposition of the oxide was removed with 0.125 per cent potassium permanganate followed by 3 per cent sodium bisulfite.

RESULTS AND DISCUSSION

Identification of Cell Types

The distinction between alpha and beta cells was facilitated by the fact that, in the rat, the alpha cells are smaller and are almost completely restricted to the edges of the islets. In Golgi preparations it is at once apparent that two cell types are present (FIGURE 1). The predominant cell is large and has a fairly elaborate Golgi apparatus. The cytoplasm of this cell type is darkened by osmic acid to a moderate degree. Arranged around the edges of the islets are a lesser number of smaller cells that have very simple and small Golgi bodies and clear cytoplasm. These cells correspond, in their location and relative numbers, to the beta cells and alpha cells, respectively, as seen in ordinary preparations stained for specific granules. The tentative identification of these cells was tested further by counterstaining some of the Golgi preparations with azo carmine, which ordinarily stains red the granules in alpha cells; in the counter-stained preparations only those cells that have been tentatively identified as alpha cells were so colored. As a further assistance in identification, the islets of alloxan-diabetic rats were studied. Due to the destruction of the beta cells, the islets of these animals consist almost entirely of alpha cells, and in Golgi preparations, as would be expected, the islets were composed predominantly of the small cells with simple Golgi bodies, already tentatively identified as alpha cells (FIGURE 2).

Studies of Beta Cell Activity

(1) *Hyperglycemia.* As a preliminary step, it was essential to ascertain how rapidly and how extensively the Golgi bodies would change in their appearance as a result of a known stimulus to their activity. There is ample evidence to show that elevation of the blood glucose level is a potent direct stimulus to the beta cells. Of the many experiments bearing on this question, two are particularly conclusive. Brown *et al.*[3] infused high concentrations of glucose into part of the arterial supply to the pancreas and observed a lowering of the blood sugar and histological evidence of stimulation in the part of the pancreas that was infused. Anderson and Long,[4] using the isolated, perfused rat pancreas, showed that the elevation of the blood glucose resulted in increased insulin levels in the perfusate. Since there is general agreement on this point, eleva-

tion of the blood glucose for varying periods of time was used as the first test of the responsiveness of the Golgi apparatus.

Sixteen experiments were carried out on both male and female rats weighing from 300 to 400 gm. Glucose was infused intravenously at rates resulting in blood levels that were continuously above 350 mg. per cent. Animals were sacrificed at intervals from 1½ to 12 hours from the beginning of the infusion. Stains for specific granules revealed almost complete degranulation of the beta cells, a finding often described previously. In the osmic acid preparation, the Golgi bodies of the beta cells of the experimental animals were somewhat larger and considerably more elaborate than those of the controls (FIGURES 3 and 4). This change was discernible at 1½ hours, well established at 4 hours, and quite marked at 12 hours. Presumably, more prolonged elevation of the blood glucose level would result in more pronounced hypertrophy of the Golgi apparatus. The only evidence supporting this assumption is afforded by the

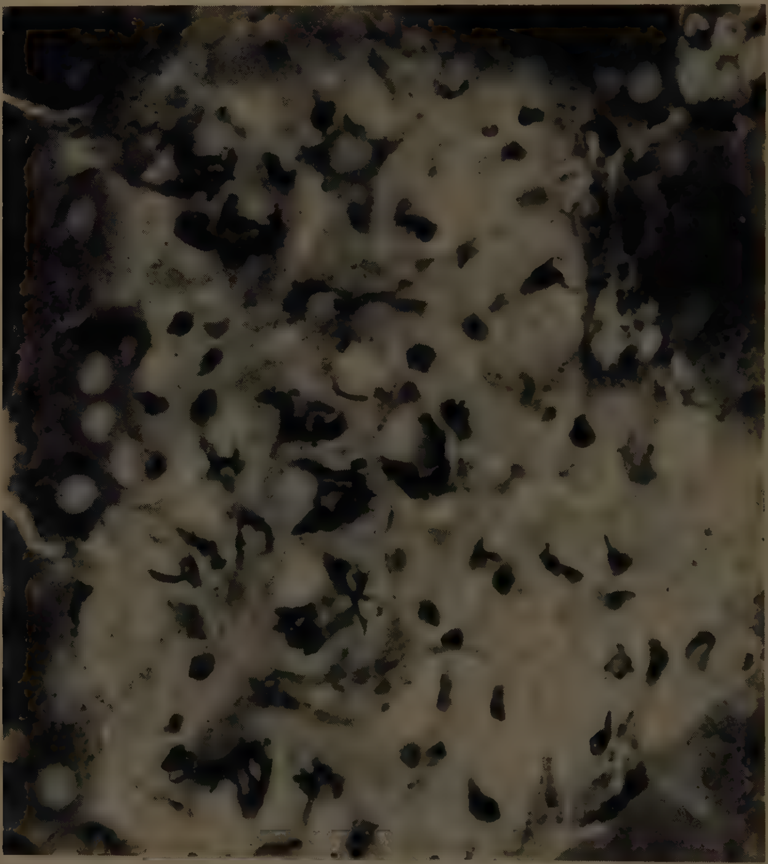

FIGURE 1. Islet of normal rat showing centrally located large cells with elaborate Golgi bodies (beta cells) and peripherally located small cells with small Golgi bodies (alpha cells). Osmic acid impregnation. ×1100.

observation that the beta cells of rats made diabetic through partial pancreatectomy show exceedingly hypertrophied Golgi bodies (FIGURE 5), presumably the result of very prolonged hyperglycemia.

(2) *Hypoglycemia.* Since hyperglycemia is a stimulus to the beta cells, it would be expected that the removal of this stimulus by reducing the blood sugar to levels below normal would result in great diminution of the activity of these cells. This expectation is supported by the work of Zunz and La Barre[5] and also by the experiments of Bennett and Sutherland,[6] who transplanted normal pancreas into the necks of pancreatectomized dogs. Bennett and Sutherland found that the blood sugar was restored to normal by this procedure following a highly reproducible curve of decline. They then observed that, in order for insulin infusions to duplicate this curve, it was necessary to reduce progressively the rate of infusion as the glucose level approached normal, indicating that the pancreas must also have reduced its rate of insulin secretion as the blood sugar fell. One would therefore expect the islets to reflect in their appearance this reduction in activity during hypoglycemia.

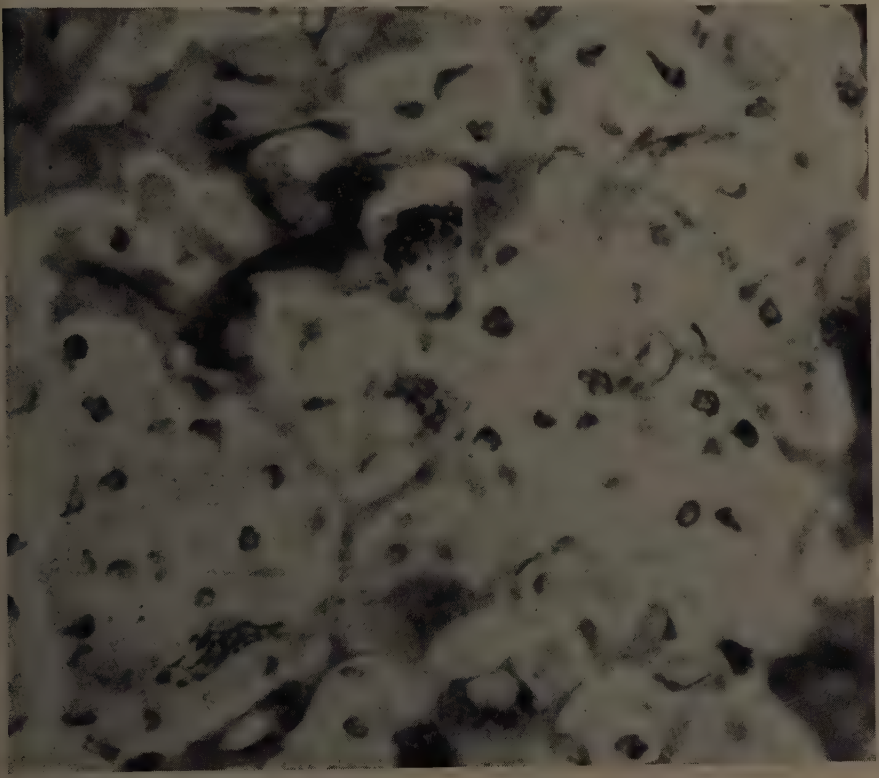

FIGURE 2. Islet of alloxan-diabetic rat. Note that islet is composed almost entirely of cells with small Golgi bodies. Osmic acid impregnation. ×1100.

Rats were made hypoglycemic with insulin for short experiments of 4 hours' duration and also for as long as 2 weeks. In the acute experiments, insulin was infused intravenously at 1.3 to 1.8 units/kg./hr., resulting in blood sugars of 40 mg. per cent or below. In the chronic experiments, 3 to 4 units of protamine zinc insulin were given subcutaneously every 12 hours for 2 weeks, resulting in blood sugars at autopsy of 37 mg. per cent or below. Four animals and their controls were used in each type of experiment. Stains for specific granules in the acute experiments revealed a normal amount of granular material in the beta cells, but in the chronic experiments the beta cells were completely degranulated. In the acute experiments the Golgi bodies were somewhat condensed in appearance, while in the chronic experiments they were very much reduced in size and complexity (FIGURE 6).

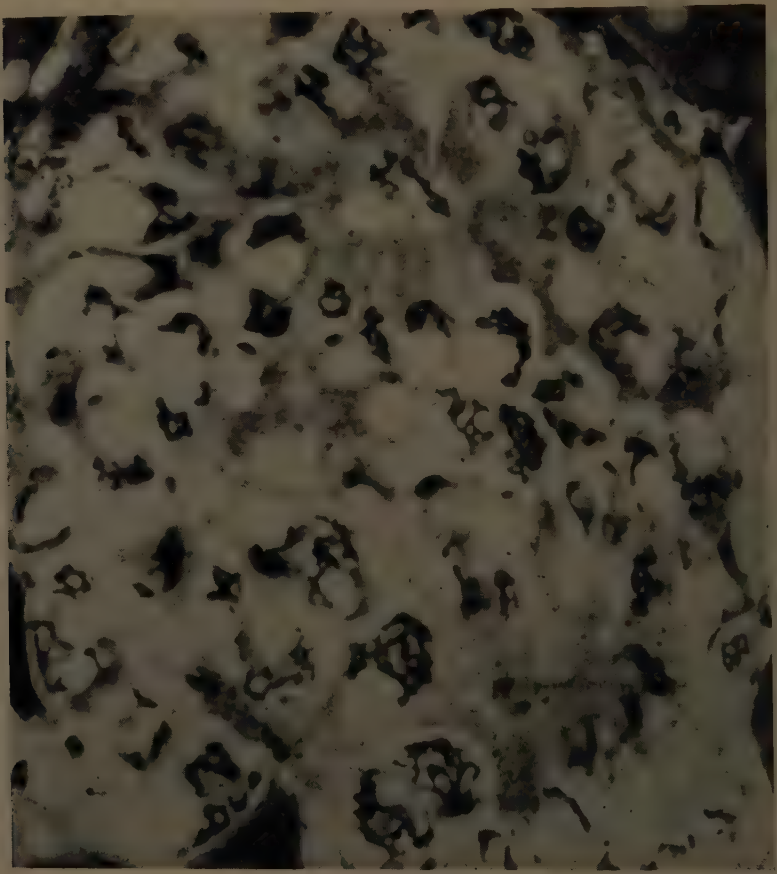

FIGURE 3. Islet of normal rat consisting almost entirely of beta cells. Compare with FIGURE 4. Osmic acid impregnation. ×1100.

It should be emphasized that in the two experimental conditions just described, the Golgi apparatus became hypertrophied during hyperglycemia and atrophic during hypoglycemia, thus apparently accurately reflecting the changes in activity known to occur. On the other hand, in both these two quite opposite functional states the specific granules of the beta cells were almost completely depleted, in the one case presumably because insulin was released as rapidly as it was produced; in the other case, because the production of insulin was greatly depressed. These findings emphasize the difficulties in interpreting changes in the granule content.

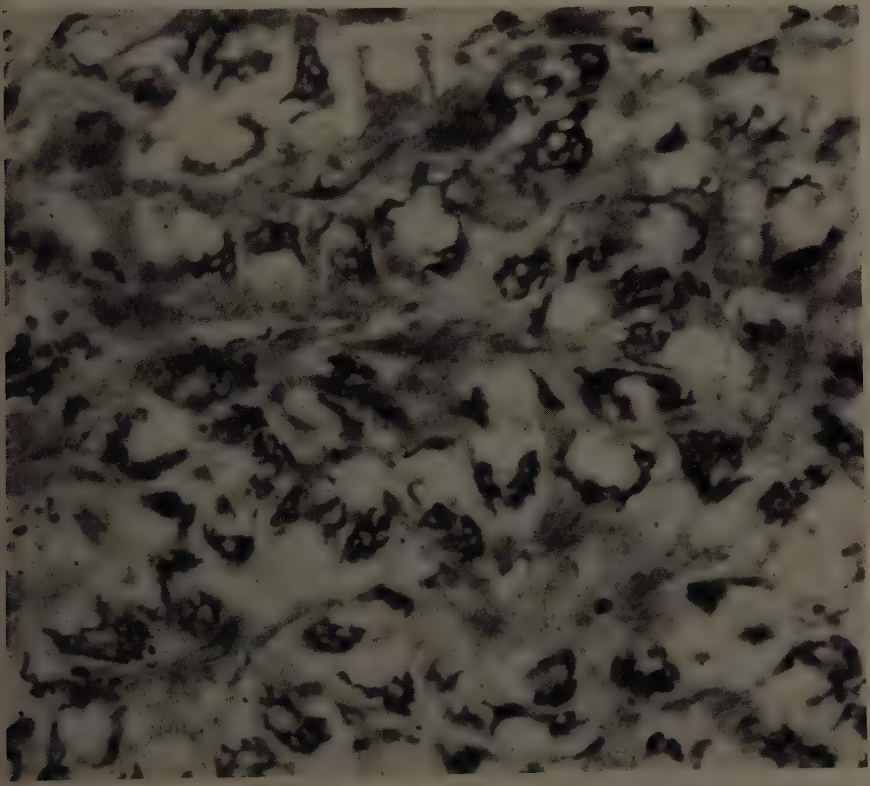

FIGURE 4. Islet of animal infused with glucose for 4 hours. Note the increase in complexity of the beta cell Golgi bodies as compared to FIGURE 3. Osmic acid impregnation. ×1100.

The simplest explanation for the diminution in function during insulin-induced hypoglycemia would be that the lowered blood sugar has removed the necessary stimulus to activity. However, the possibility that insulin itself may directly inhibit beta cell activity is suggested by the work of Hausberger and Ramsay,[7] who reported that the simultaneous administration of large doses of insulin, together with sufficient glucose to produce sustained hyperglycemia, resulted in abolition of the usual histological changes in the islets that resulted from the administration of glucose alone. This point seemed of sufficient interest to re-examine. Accordingly, 13 rats were treated with 3 units of protamine zinc insulin subcutaneously every 12 hours for 2 or 3 days, and one half of them were subsequently infused with 1 unit of HGF-free insulin (Lilly) per hour along with sufficient glucose to maintain blood sugar levels above 300 mg. per cent for 4 hours. Specific granule stains of islets of the animals receiving insulin alone showed a somewhat heavier degree of beta cell granulation than is seen in normal controls. The islets of the animals that received both insulin and glucose showed extensive degranulation of the beta cells. The Golgi bodies of the beta cells of those animals that received both glucose and insulin were noticeably more elaborate than those treated with insulin only (FIGURE 7). Since the presence of large amounts of insulin did not interfere with the response of the beta cells to hyperglycemia, one may conclude that it is the level of sugar rather than insulin in the blood that controls the function of the beta cells.

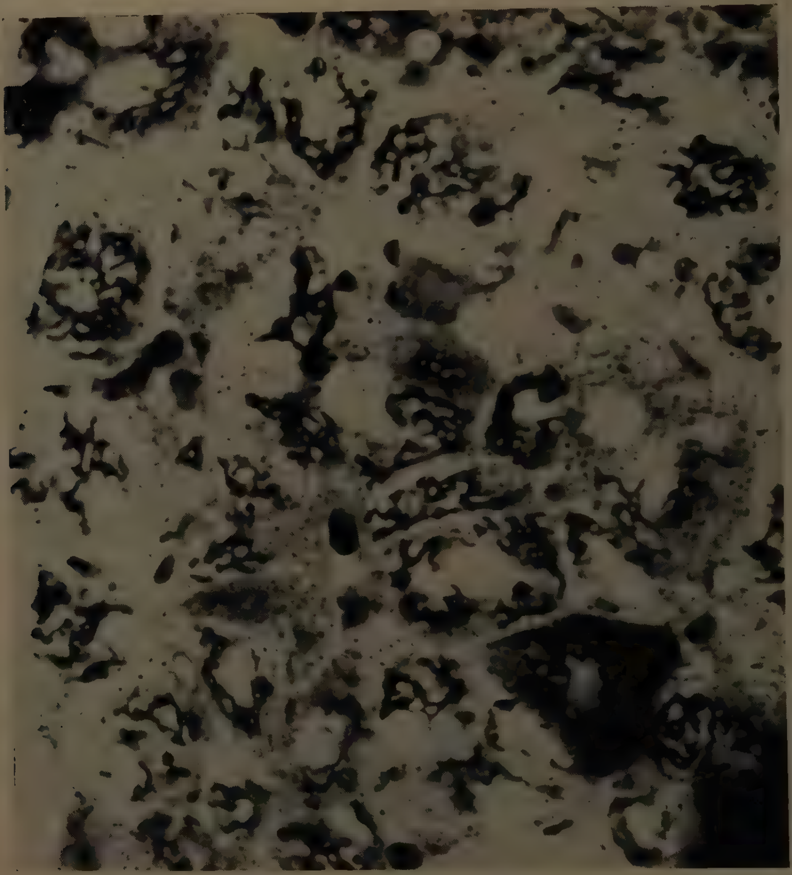

FIGURE 5. Islet of partially pancreatectomized, diabetic rat 60 days after operation. Note the extensive development of Golgi bodies in beta cells. Osmic acid impregnation. ×1100.

(3) *Influence of the pituitary.* Many years ago, a pancreotropic effect of the

pituitary on the islets was proposed by Anselmino and his co-workers[8, 9] on the basis of the finding that injection of anterior pituitary extracts resulted in hypertrophy of the islets. This effect was ultimately confirmed by Richardson and Young,[10] and has been shown to be duplicated by highly purified preparations of growth hormone.[11] If the pituitary exerts a stimulatory effect upon the islets, one would expect hypophysectomy to result in some degree of hypofunction and consequent atrophy. However, adequate estimations of islet mass have revealed only suggestions of such a change[11, 12] and the histology has been reported to be normal.[13] It seemed worthwhile to reinvestigate this point.

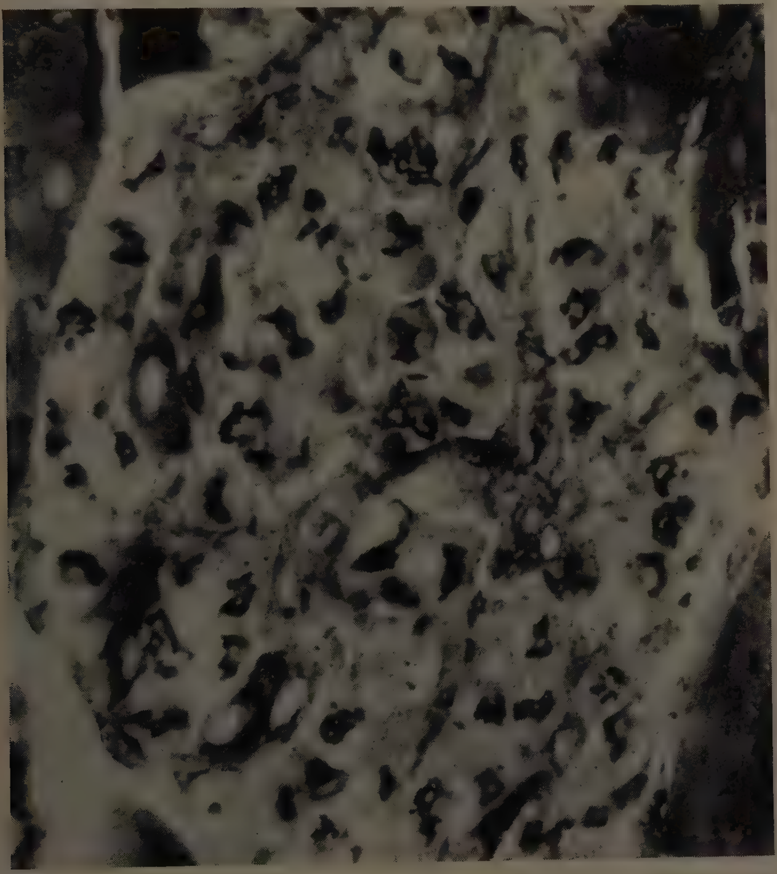

FIGURE 6. Islet of rat treated with insulin for 2 weeks. Note the reduction in complexity of Golgi bodies. Compare with FIGURE 3. Osmic acid impregnation. ×1100.

Twelve rats were hypophysectomized by the parapharyngeal approach at 40 days of age and were then placed in individual cages so that their food intake could be measured. Twelve normal rats of the same age and sex were also placed in individual cages, and each was pair-fed with one of the hypophysectomized rats. After 14 days both groups were autopsied, together with a group of ad libitum-fed controls, and the completeness of operation was checked by examination of the operative site. Specific stains for granules showed islets of normal appearance and granule content in both hypophysectomized rats and their pair-fed controls, with the exception that the beta cells appeared smaller in the islets of the hypophysectomized rats. Golgi preparations, however, revealed a very marked reduction in the size and complexity of the Golgi bodies in the beta cells of the hypophysectomized rats, which was much more pronounced than the very moderate change seen in the islets of the pair-fed controls (FIGURES 8 and 9). Therefore the beta cells in the hypophysectomized rat appear to be reduced in function to a greater degree than can be explained by reduction in food intake alone, which suggests that this change may have resulted from the absence of one or more stimulatory hormones. Support for the concept that the beta cells are functioning at a low level in the hypophysec-

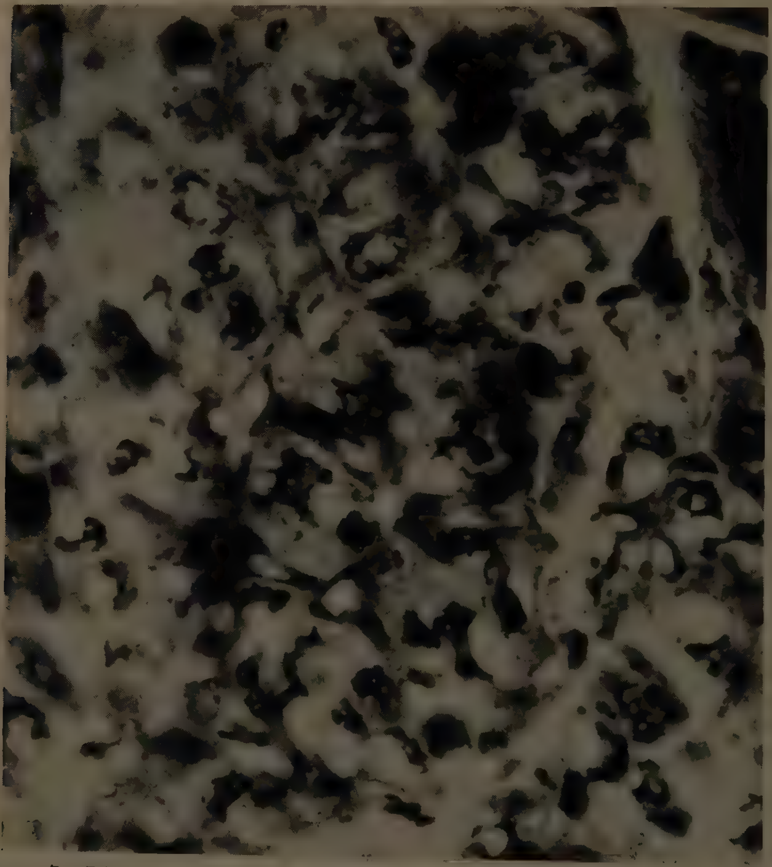

FIGURE 7. Islet of animal that was treated with insulin and glucose. Beta cell Golgi bodies are more elaborate as compared with FIGURE 6. Osmic acid impregnation. ×1100.

tomized animal is afforded by Randle,[14] who has found lowering of blood insulin levels following hypophysectomy. On the other hand, an investigation of the ability of the pancreas of the hypophysectomized dog to respond to the stimulus of hyperglycemia was carried out by Houssay *et al.*,[15] who found that, when such a pancreas was transplanted into the neck of a diabetic dog, it restored the blood sugar to normal in the same length of time as did the pancreas of a normal dog. However, there is some suggestion in their data that the initial rate of decline in the blood sugar may have been slower when the pancreas of the hypophysectomized dog was used, even though the same final sugar level was attained.

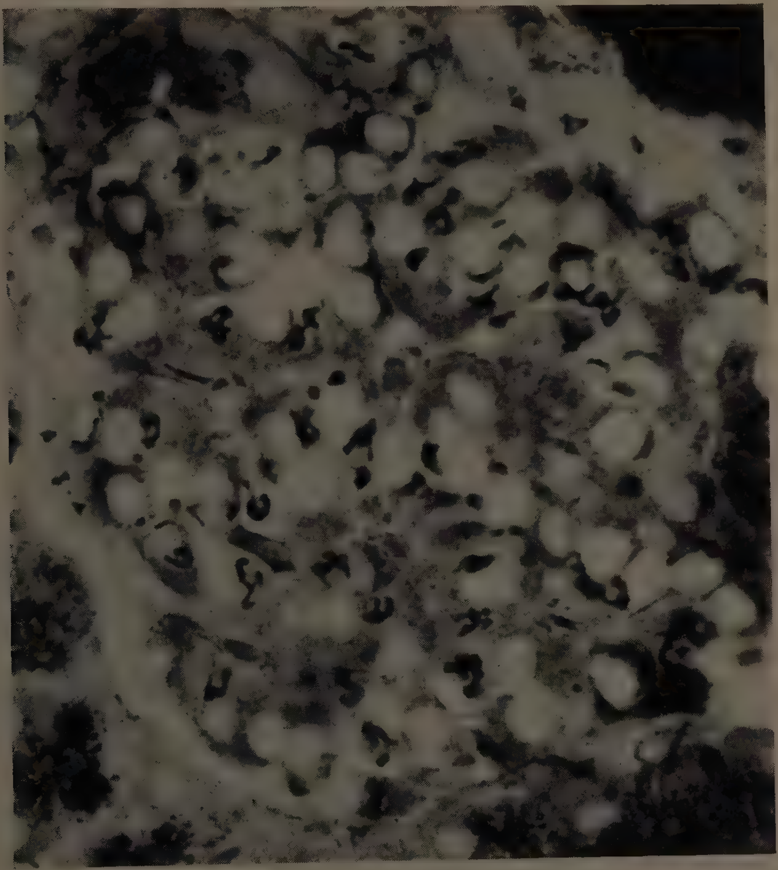

FIGURE 8. Islet of animal hypophysectomized 14 days previously. Note the extreme reduction in complexity of Golgi bodies of beta cells. Compare with FIGURE 3, an ad libitum-fed control, and with FIGURE 9, a pair-fed control. Osmic acid impregnation. ×1100.

Most of the available evidence suggests that growth hormone stimulates the islets, probably directly, although in only a few cases has it been possible to observe this effect without a concomitant increase in blood sugar. For example, Randle[14] reported that the blood of cats treated with growth hormone contained increased insulin activity, but the blood sugars were also slightly elevated. On the other hand, Anselmino and Hoffmann[16] long ago showed that pituitary extracts would produce a prompt decline in the blood sugar of the intact dog, but would produce only elevation in the blood sugar of dogs that had been previously pancreatectomized and allowed to recover thoroughly from the effects of the operation. Moreover, Milman and Russell[17] also observed a very marked decline in the blood sugar levels of hypophysectomized or adrenalectomized rats given growth hormone. Therefore, it seemed of interest to determine whether stimulation of the beta cells by growth hormone could be detected histologically.

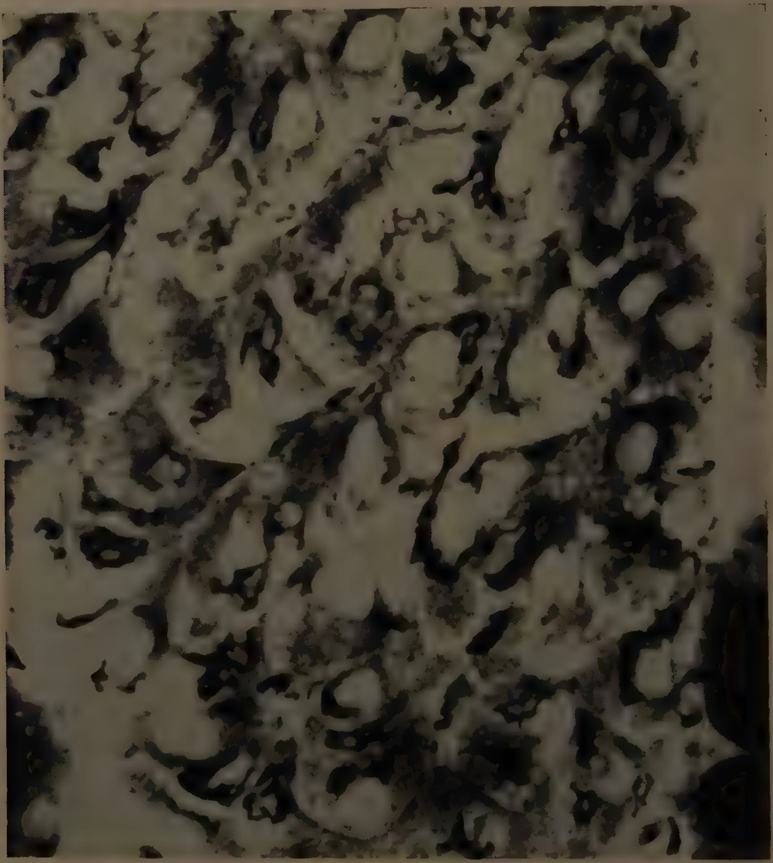

FIGURE 9. Islet of normal rat whose food intake was restricted to that of animal whose islet is shown in FIGURE 8. Note moderate reduction in complexity of beta cell Golgi bodies. Osmic acid impregnation. ×1100.

Four hypophysectomized rats, prepared as above, were treated for 4 days with 2.5 mg. of growth hormone (prepared by the method of Wilhelmi *et al.*[18]) per day intraperitoneally. Another group of hypophysectomized rats was similarly treated, but their food intake was restricted to that of the untreated hypophysectomized group in an effort to eliminate the variable of changes in food consumption. Unfortunately, all members of this group died, presumably of hypoglycemia. The treated hypophysectomized animals fed ad libitum all showed restoration of the beta cell Golgi bodies toward normal (FIGURE 10). However, their increased food intake makes interpretation of this experiment difficult. Therefore, an experiment was undertaken with normal rats under conditions that, it was hoped, would prevent either alterations in food intake or changes in blood sugar levels from influencing the outcome. Four animals were treated with 3 units of protamine zinc insulin subcutaneously every 12 hours for 2 weeks, while a second group of 4 animals was treated with the same dose of insulin and also with 0.5 mg. of purified growth hormone subcutaneously every 12 hours for 2 weeks. At autopsy, the blood sugar values of the insulin-treated animals averaged 54 mg. per cent, and those of the insulin- and growth

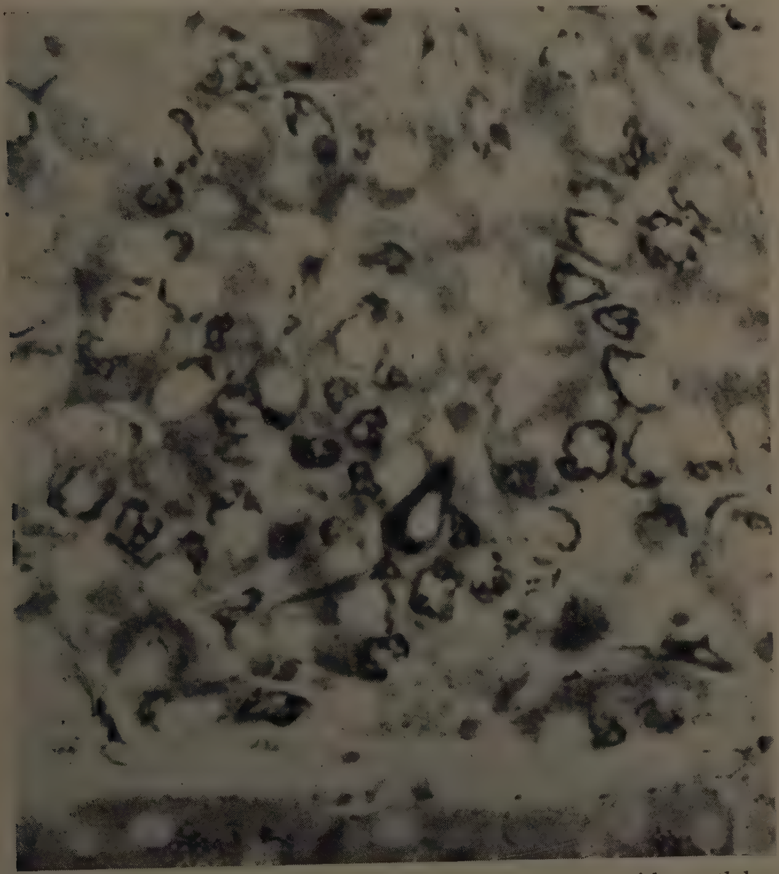

FIGURE 10. Islet of hypophysectomized animal treated for 4 days with growth hormone. Note the return of Golgi bodies toward normal in appearance. Osmic acid impregnation. ×1100.

hormone-treated animals averaged 52 mg. per cent. Study of the aldehyde-fuchsin preparations revealed the usual degranulation in the insulin-treated group but, in the group treated with both hormones, the amount of beta cell granulation was restored toward normal. In the Golgi preparations, atrophy of the Golgi bodies was seen in the insulin-treated group as previously described, but in the group treated with insulin and growth hormone the Golgi bodies were significantly larger and more elaborate (FIGURE 11). Since the blood sugars were presumably at hypoglycemic levels during the 2-week period, the observed stimulation of the Golgi bodies must have resulted from direct stimulation of the beta cells by the growth hormone itself rather than by any secondary effect on the blood sugar.

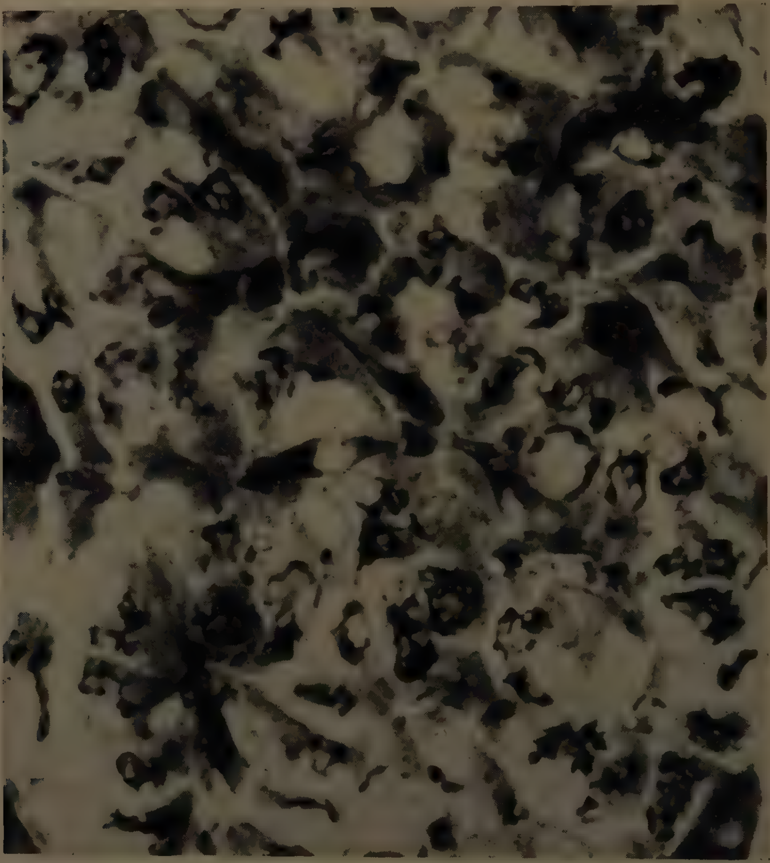

FIGURE 11. Animal treated with insulin and growth hormone for 2 weeks. Compare with FIGURE 6. Note the increase in complexity of beta cell Golgi bodies compared to those of animal treated with insulin alone. Osmic acid impregnation. ×1100.

Studies of Alpha Cell Activity

Very strong evidence connects the alpha cells and the presence of extractable glucagon in the pancreas. Neither atrophy of the acinar tissue produced by ligation of the duct nor the destruction of the beta cells by alloxan produced any diminution in the amount of glucagon extractable from the pancreas;[19] moreover, the uncinate process of the dog pancreas, a region almost devoid of alpha cells, was found to have an exceedingly low glucagon content.[20, 21] However, before glucagon can be established as a hormone, it must be shown to be secreted in response to physiological stimuli. In cross-circulation experiments, Foà *et al.*[22] have reported that pancreatic venous blood from a hypoglycemic dog contained a substance that induced hyperglycemia in a recipient animal, while mesenteric vein blood from the same animal did not. Moreover, Bornstein *et al.*[23] found that pancreatic venous blood from cats treated with growth hormone also contained a substance producing hyperglycemia. Fodden and Read[24] also demonstrated this effect following administration of growth hormone and ACTH. These observations have led to histological studies of the alpha cells with the expectation that the changes in function believed to occur would be reflected in their appearance. The effect of chronic insulin treatment

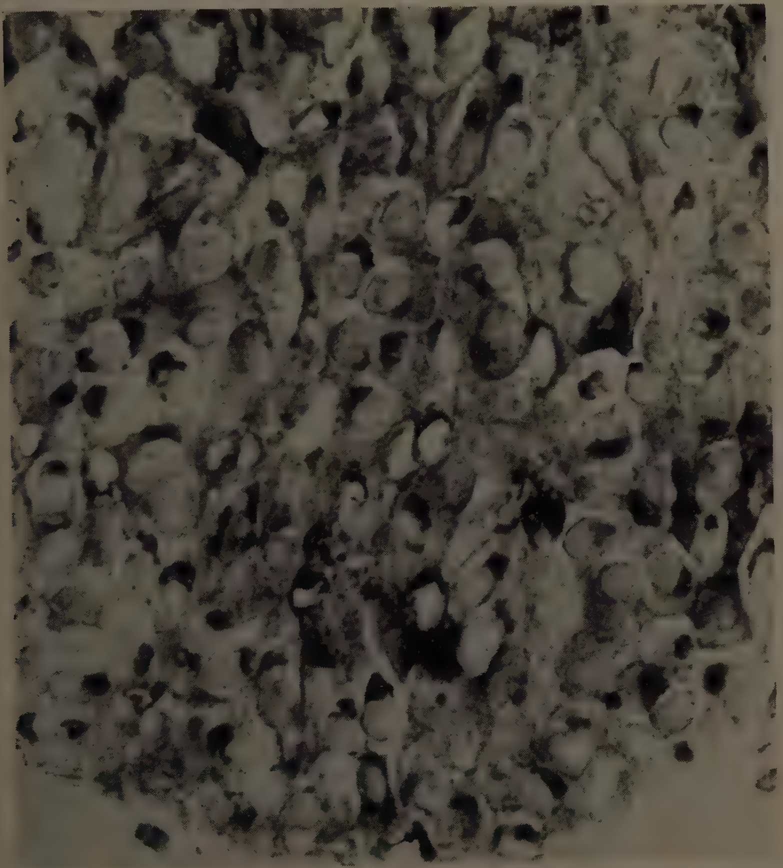

FIGURE 12. Islet of alloxan-diabetic rat treated with insulin for 2 weeks. Islet composed almost entirely of alpha cells. Compare with FIGURE 2. Alpha cell Golgi bodies unchanged by prolonged hypoglycemia. Osmic acid impregnation. ×1100.

has been studied, with a variety of results. Latta and Harvey[25] treated mice with insulin for 75 days and observed no changes in the alpha cells. Hagemann[26] reported that chronic hypoglycemia increased the alpha-to-beta-cell ratio, but this finding could be accounted for on the basis of a reduction in the size and number of the beta cells. Recently, Hökfelt and Hultquist[27] have reported degranulation of the alpha cells in rabbits as a result of insulin treatment. As a result of the divergent findings, studies of the Golgi bodies in the alpha cells of animals chronically treated with insulin were undertaken.

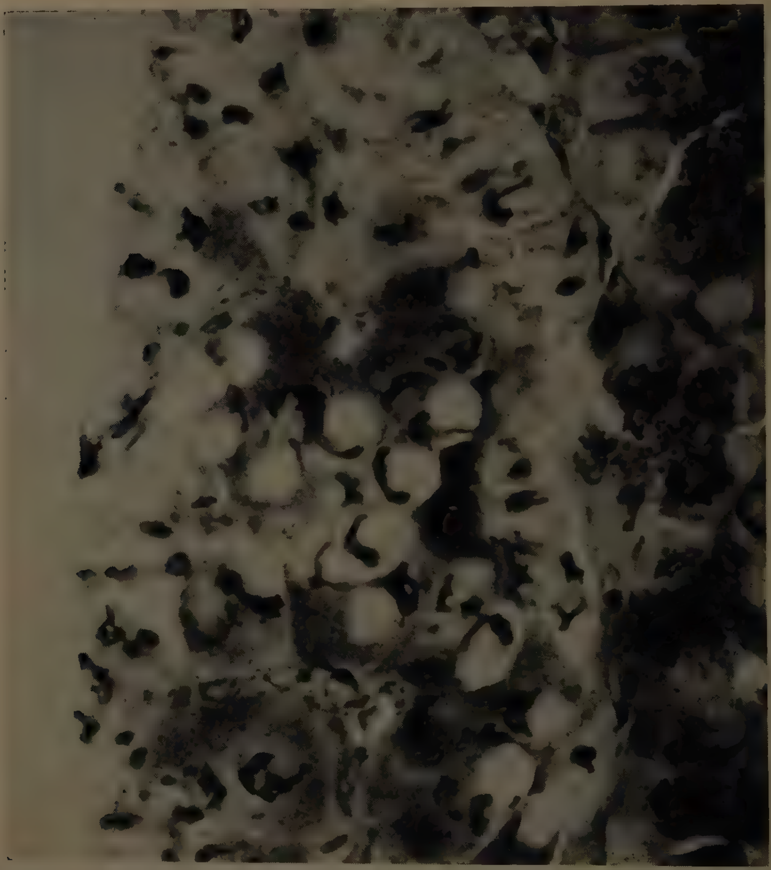

FIGURE 13. Islet of animal hypophysectomized 14 days previously. Cells with light cytoplasm are alpha cells. Compare with FIGURE 1. Golgi bodies appear unchanged. Osmic acid impregnation. ×1100.

The alpha cells were studied in normal animals treated with insulin in the experiments described above. In no case did either the granulation or the Golgi bodies change in any way as a result of acute or chronic hypoglycemia. Because of the relative sparseness of the alpha cells in normal animals, 5 alloxan-diabetic rats were made hypoglycemic for 4 hours, and 4 diabetic rats were maintained in a hypoglycemic state for 2 weeks as outlined above. In both groups of animals the granule content of the alpha cells was indistinguishable from the normal; furthermore, the Golgi bodies (FIGURE 12) appeared entirely unchanged.

The effects of changes in pituitary function upon the histology of the alpha cells have been studied quite extensively. Ferner *et al.*[28] have claimed that hypophysectomy resulted in reduced numbers of alpha cells, while growth hormone treatment increased their numbers. However, it was later pointed out[13] that their staining method was unreliable, and further studies have revealed no change in the alpha cells following hypophysectomy, although very prolonged treatment with large doses of growth hormone resulted in an increase followed by a decrease in numbers. Therefore, the alpha cells were carefully studied in the experiments on hypophysectomized and growth hormone-treated animals outlined above. Both the content of granular material and the appearance of the Golgi bodies were entirely unchanged in all cases (FIGURE 13).

The immutability of the alpha cells in at least the mouse and rat is very curious. Since some change as a result of hypoglycemia has recently been reported in rabbits, and since the studies showing apparent glucagon secretion have been performed in dogs and cats, it is possible that mice and rats are unfavorable test animals and possibly do not produce glucagon. Some of the experiments outlined above are being repeated in rabbits. On the other hand, another possible explanation is afforded by the work of Sirek *et al*,[29] who have confirmed the finding that the administration of growth hormone causes the appearance in the pancreaticoduodenal vein blood of the dog of a material that induces hyperglycemia in recipient dogs. However, this material was still present following growth hormone treatment of pancreatectomized dogs; moreover, its effect was blocked by pretreatment of the recipient animal with dihydroergotamine, a substance that blocks the effect of epinephrine, but not of glucagon.[30] These findings cast doubt upon the identity of the material found in the blood in the experiments cited above, and reopen the entire question of whether glucagon is actually secreted. If it is not, the unchanging aspect of the alpha cells is quite understandable.

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MORPHOLOGIC REFLECTION OF INCREASING INSULINOGENESIS DURING CHRONIC HYPERGLYCEMIA IN THE RABBIT

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In physiological studies of the effect on the blood sugar of various hyperglycemic agents, it is generally ignored that the pancreas has considerable functional reserve and is not static in its insulinogenic activity.[1] This is borne out by the fact that many acromegalic patients,[2] as well as individuals with endogenous or exogenous adrenal steroid excess,[3] do not develop hyperglycemia. Similarly, it has been observed that in some animal species there is gradual adaptation to the diabetogenic action of exogenously administered hyperglycemic agents. Thus, the temporary diabetes induced by growth hormone or by adrenal steroids administered alone or together with glucagon is characterized by the gradual development of glycosuria, which then slowly declines only to become re-established on increasing the hormone dosage.[4-11] The fact that adaptation to a diabetogenic stimulus is frequently overcome when the pancreatic reserve is reduced, either surgically[12-15] or chemically,[16] suggests that increased insulinogenesis is the mechanism involved.

The present study demonstrates that the rabbit gradually adapts to prolonged hormonally induced hyperglycemia, and that the pancreatic islets show concomitant morphologic alterations that reflect increasing insulinogenesis.

Material and Methods

The study was carried out on 86 New Zealand white rabbits, of both sexes, weighing between 2500 to 4500 gm., divided into 4 groups. Group 1 comprised 36 rabbits, each of which received cortisone acetate (4 mg./kg.) intramuscularly daily during the first 21 days, and then 8 mg./kg. daily for up to 60 days. Group 2 consisted of 18 animals that received subcutaneously crystalline glucagon suspended in corn oil (1 mg./kg.) 3 times daily, at 10 A.M., 4:30 P.M. and 12 midnight and, in addition, cortisone acetate in aqueous suspension (1 mg./kg.) intramuscularly daily. Group 3 consisted of 20 rabbits that received cortisone acetate (1 mg./kg.) daily intramuscularly together with adrenaline in oil (2.0 mg.) subcutaneously 3 times daily, as in group 2, for up to 104 days. Group 4 consisted of 12 untreated rabbits that served as controls.

Each animal was kept in a metabolic cage and received Purina Lab Chow and water ad libitum, as well as 200,000 units of procaine penicillin G and 0.25 gm. of dihydrostreptomycin sulfate (Combiotic*) twice weekly. Blood was withdrawn daily from the marginal ear vein at approximately 9:30 A.M. and 1:30 P.M. The blood sugars were determined by the Nelson-Somogyi micromethod.[17] All animals were sacrificed by overdosage with barbiturates, and pancreatic tissue was placed immediately into Zenker formol solution (20 per cent) for histological study. After paraffin embedding, the slides were stained by the following methods: iron hematoxylin, phosphotungstic hematoxylin, modified Masson trichrome,[18] modified aldehyde-fuchsin trichrome,[19] a modification of Gomori's chrom alum hematoxylin,[20] and the periodic acid Schiff trichrome controlled by diastase digestion for glycogen identification.[21]

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* Chas. Pfizer & Co., Brooklyn, N. Y.

Results

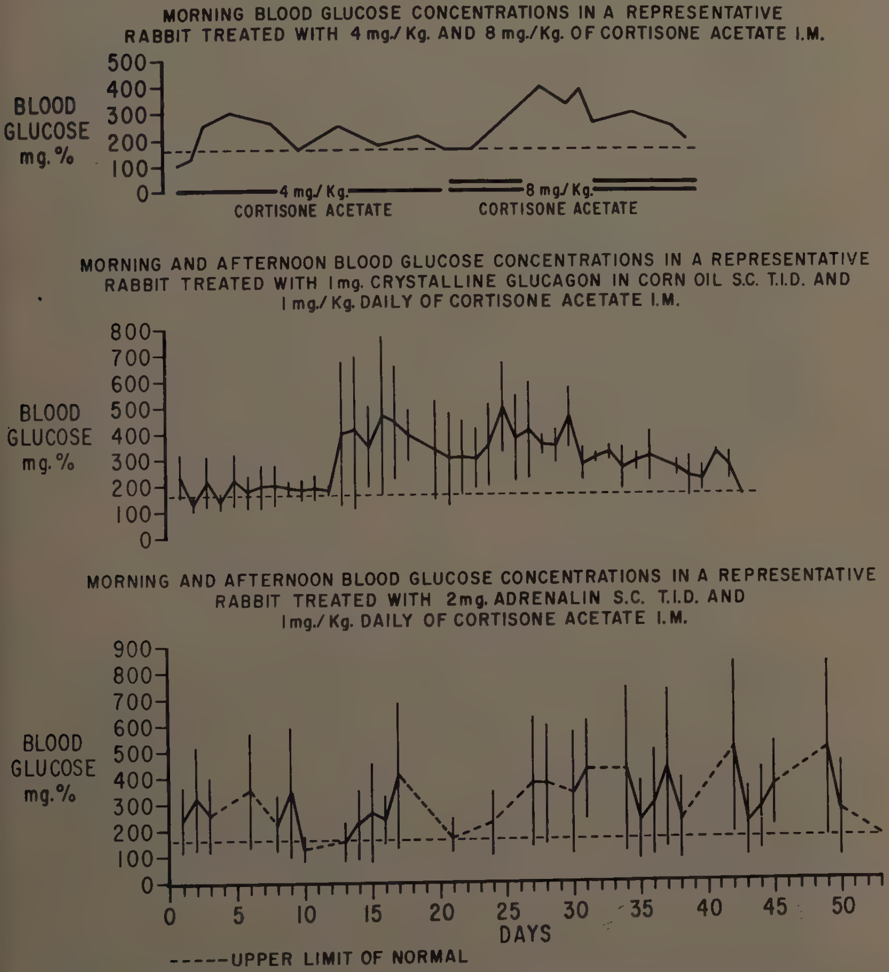
The untreated control rabbits showed no significant differences between the morning and afternoon blood sugar values, which varied between 93 and 158 mg. per cent, a fasting blood sugar value higher than 160 mg. per cent was therefore considered a significant elevation. The animals that received large doses of cortisone alone showed, in general, 2 peaks of hyperglycemia: the initial peak blood sugar level was reached between the third and fifth days after starting treatment with cortisone (4 mg./kg.). After that time the blood sugar tended to decline, reaching its nadir within 3 weeks. On doubling the dose of cortisone at 21 days, a secondary rise of the blood sugar level was usually obtained, which eventually again tended to decline to normal. With these cortisone dosages the maximum blood sugar levels varied up to 375 mg. per cent (FIGURE 1, *top*).

The greatest degree and duration of hyperglycemia was developed in those rabbits treated simultaneously with cortisone and glucagon. In these animals the morning dose of glucagon produced a marked hyperglycemic response that was fairly well sustained until the afternoon. The morning values were frequently within normal limits during the early stage of treatment; thereafter they increased to as much as 400 mg. per cent. The afternoon values were elevated from the beginning. This elevation became extreme as treatment continued, with occasional values reaching 830 mg. per cent. After 6 weeks the morning glycemic levels were frequently less than they had been prior to that time, and tended to return to normal (FIGURE 1, *center*).

The animals treated with adrenaline plus cortisone showed intermittent hyperglycemia. The morning blood sugar values varied between 82 and 120 mg. per cent, and rarely exceeded upper normal levels. The afternoon values were markedly increased, and ranged up to 840 mg. per cent (FIGURE 1, *bottom*).

The sequential morphologic alterations of the pancreas of all hormonally treated animals were quite similar. Within two days the degranulation of beta cells was observed which, concomitantly with increasing blood sugar levels, became very marked or even complete (FIGURE 2). The beta cells were generally larger than normal, as were their nuclei, which were more ovoid and had more sparsely distributed chromatin than does the normal beta cell. Increased mitotic activity of the beta cells was noted between the third and eleventh days (FIGURE 3). Glycogenic vacuolization appeared first in ductular epithelium and then also in the beta cells, and its extent was related to the severity and duration of diabetes. It is of interest that the rabbits treated with adrenaline plus cortisone, which developed only intermittent hyperglycemia, showed extensive ductular glycogen deposition; however, except in one case, no beta cell glycogen infiltration was found.

In animals in which prolonged hyperglycemia was maintained, most ductular epithelial cells were vacuolated because of glycogen infiltration, but additional nonvacuolated hyperplastic ductules were found that were intrainsular or contiguous with islets. In occasional instances, primitive beta cells were seen originating from this type of hyperplastic ductular epithelium (FIGURE 4).


MORNING BLOOD GLUCOSE CONCENTRATIONS IN A REPRESENTATIVE RABBIT TREATED WITH 4 mg./Kg. AND 8 mg./Kg. OF CORTISONE ACETATE I.M.

MORNING AND AFTERNOON BLOOD GLUCOSE CONCENTRATIONS IN A REPRESENTATIVE RABBIT TREATED WITH 1 mg. CRYSTALLINE GLUCAGON IN CORN OIL S.C. T.I.D. AND 1 mg./Kg. DAILY OF CORTISONE ACETATE I.M.

MORNING AND AFTERNOON BLOOD GLUCOSE CONCENTRATIONS IN A REPRESENTATIVE RABBIT TREATED WITH 2mg. ADRENALIN S.C. T.I.D. AND 1 mg./Kg. DAILY OF CORTISONE ACETATE I.M.

FIGURE 1. Top, morning blood sugar levels of a rabbit treated with cortisone acetate (4 mg./kg. followed by 8 mg./kg.); center, morning and afternoon blood sugar levels and their average in a rabbit treated with crystalline glucagon (1 mg. in oil S.C., t.i.d.) and cortisone acetate (1 mg./kg. I.M., daily); and bottom, of a rabbit treated with 2 mg. adrenaline in oil S.C. t.i.d. and cortisone acetate (1 mg./kg. I.M., daily).

These cells showed large nuclei with abundant opaque somewhat reticulated cytoplasm. Also seen within islets were young beta cells with sparse diffuse aldehyde-fuchsin-positive granules (FIGURE 5). These cells were differentiated from adjacent older beta cells by the fact that the latter were considerably smaller and were degranulated, showing aldehyde-fuchsin-positive cell borders

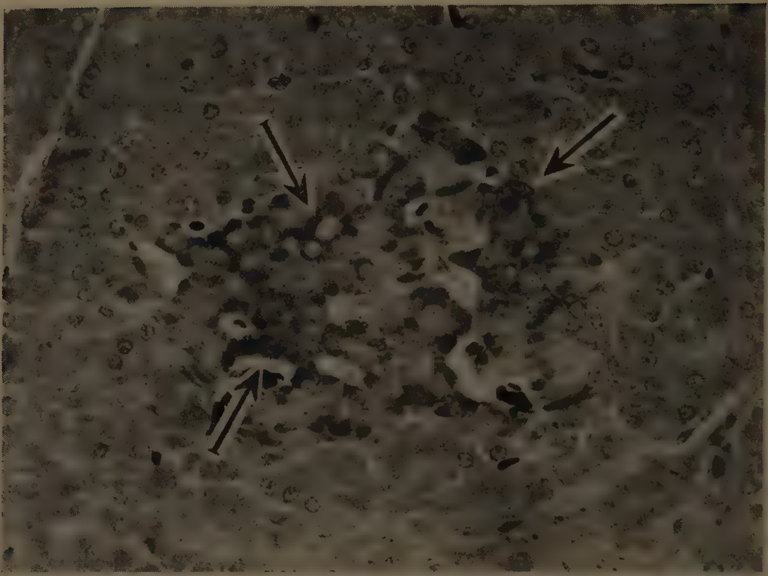


Figure 2. Islet of untreated rabbit showing well-granulated beta cells (*arrows*). Aldehyde-fuchsin trichrome stain. ×378.

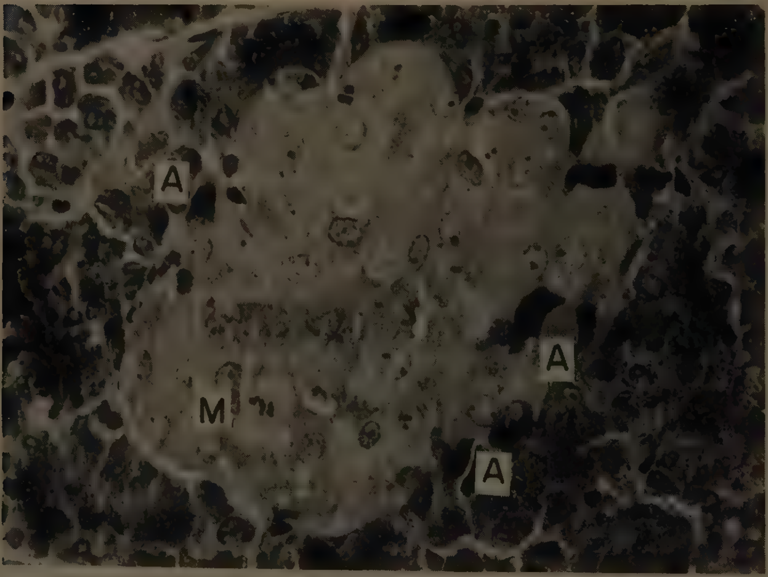


Figure 3. Pancreas of a rabbit treated with cortisone acetate (4 mg./kg.) for 7 days. There is a mitotic figure (M) and extensive B cell degranulation. The alpha cells appear dark (A). Periodic acid Schiff trichrome stain. ×500.

(FIGURE 5). Occasional well-granulated beta cells were seen which were either intrainsular or intraductular (FIGURE 6) in location, despite the fact that the beta cells in the rest of the pancreas were in general extensively degranulated. In the animals treated for the longest periods of time the islets were markedly hyperplastic, with irregular contours, and visual estimates of their volume showed that they were markedly increased over that seen in normal rabbits (FIGURE 7). Occasionally an acinar arrangement of beta cells was seen in which the cells surrounded a central lumen that contained Schiff-positive material. Additional morphologic changes in the pancreas unrelated to the chronic hyperglycemia were also found. The animals that received glucagon and cortisone showed marked decrease of identifiable alpha cells, as described elsewhere.[10] With the larger dosages of cortisone alone, but also to a lesser extent in the other two groups, there was occasional intraductular inspissation of secretion, ductular dilatation, and occasional pancreatic lobules that consisted of proliferating dilated ductules and dedifferentiated acinar tissue with comparatively intact islets.

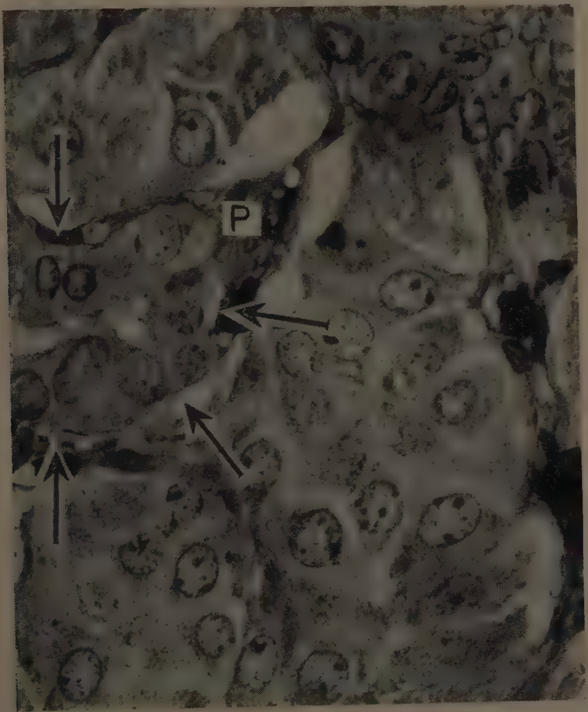


FIGURE 4. Pancreas of rabbit treated with glucagon and cortisone for 106 days, showing an intrainsular proliferating ductule (*arrows*) with a primitive beta cell (P) originating from it. Periodic acid Schiff trichrome stain. ×960.

Discussion

Prolonged administration of diabetogenic hormones in the rabbit does not produce permanent diabetes. The degree of diabetes wanes as treatment is prolonged, despite the maintenance of hormonal dosage. This decline of the hyperglycemia is thought to result from increased insulin release from the pancreas, which is reflected in the morphologic changes in it. Thus, soon after the initiation of hormonal treatment, there was degranulation and increased mitotic activity of beta cells. In the animals treated for prolonged periods of time there was marked islet hyperplasia, and it was possible to trace new beta cell formation from ductular epithelium.

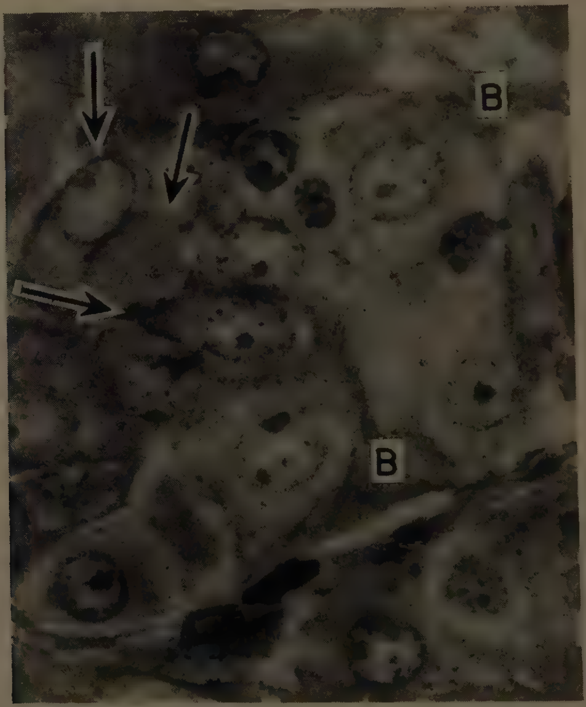


FIGURE 5. Pancreas of same rabbit as in FIGURE 4, showing large young beta cells (B) with sparse aldehyde-fuchsin-positive granules. The adjacent older beta cells are smaller, degranulated, and have aldehyde-fuchsin-positive cell borders (*arrows*). Aldehyde-fuchsin trichrome stain. ×1600.

Most workers[22-25] have considered beta cell degranulation and hyperplasia, as well as neogenesis of beta cells, to be compensatory mechanisms for hyperglycemia. However, no unanimity of opinion exists as to the origin of newly formed beta cells. In the embryo, beta cells have been stated to develop either by differentiation from primitive ductular epithelium[26] or by transformation from acinar cells.[27] Under various conditions in postfetal life, neogenesis of beta cells has been claimed to occur from acinar cells and from other islet cell types as well as from ductular epithelium.[23, 28-32] The principal evidence for the former type of transformation has been either geographic contiguity, the supposed presence within the same cell of different types of intracellular granules, or an acinar arrangement of beta cells.[23, 29, 30] However, it is generally acknowledged that mature cells reproduce only themselves, while primitive cell types may differentiate in various directions. In the study presented here, no evidence of beta cell neogenesis from acinar cells, alpha cells, or delta cells was found. This is in agreement with the observation that apparent acino-insular transformation, as well as the interchangeability of various islet cell types, is an artifact, either due to superimposition of two different cell types or to a confusion of mitochrondria with intracytoplasmic secretory granules.[27] Moreover, the acinar arrangement of beta cells around a central lumen containing Schiff-positive material could also support the hypothesis that these cells are derived through transformation of ductular epithelium.[33]

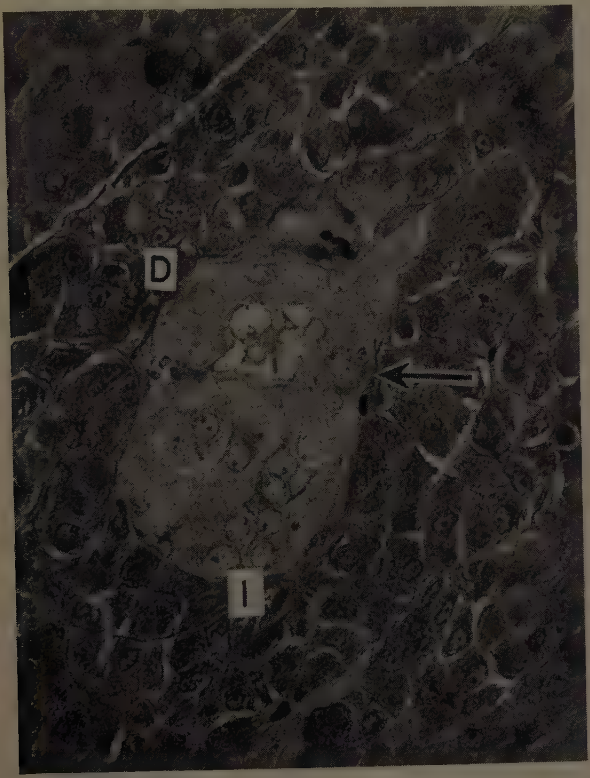


FIGURE 6. Pancreas of rabbit treated with adrenaline and cortisone for 104 days, showing islet (I) apparently originating from a hyperplastic ductule (D). The beta cells are degranulated except for a single newly formed well-granulated intraductular beta cell (*arrow*). Aldehyde-fuchsin stain. ×500.

The experimental finding that chronic hyperglycemia reflects itself in morphologic changes of the beta cells indicative of increased insulinogenesis might have an analogue in the human pancreas. Various workers have suggested that there may be hyperplasia of islet tissue as a concomitant of some form of diabetes.[34, 35] Also, it is well known that hyperplasia of the islets occurs in infants of prediabetic mothers.[36-38] In both instances this is probably a compensatory response. Furthermore, only a very small percentage of individuals treated with adrenal steroids develops diabetes. It has also been shown that pronounced diabetes due to adrenocortical tumors or pheochromocytoma may be cured by surgery. These observations suggest that in the human there is adaptation by the pancreas to diabetogenic stimuli, and diabetes may occur in man without causing damage to the pancreas sufficient to maintain the diabetic state after the offending lesion is removed.

FIGURE 7. Pancreas of rabbit treated with cortisone acetate (1 mg./kg.) daily and 1 mg. crystalline glucagon t.i.d. for 106 days, showing marked islet hyperplasia. A newly formed islet is loosely attached to a hyperplastic ductule (*arrow*). Aldehyde-fuchsin stain. ×63.

Summary

A study was conducted on the sequential morphologic changes in the pancreas of rabbits during administration of various diabetogenic hormones. Animals were treated either with large doses of cortisone alone or with subdiabetogenic dosages of cortisone together with glucagon or adrenaline. The animals developed varying degrees of hyperglycemia but, in general, as treatment was prolonged, hyperglycemia tended to wane despite maintenance or even increase of the hormone dosage level.

The earliest morphologic change was degranulation of the beta cells, which gradually became complete. Within the first 3 to 11 days after treatment was

started, increased mitotic activity of beta cells was seen. In addition, glycogen was found within the ductular epithelium. In severely diabetic animals this was followed by the deposition of glycogen within beta cells. In no instance was there any evidence of destruction of beta cells. With prolongation of treatment for up to 104 days, new beta cell formation from ductular epithelium could be followed. This eventually resulted in a marked increase in the volume of islet tissue. New beta cell formation was first evidenced by the appearance within ductules of large cells with opaque somewhat reticulated cytoplasm. Subsequently, occasional cells were found with sparse, diffusely distributed intracytoplasmic aldehyde-fuchsin-positive granules. At a later stage newly formed mature individual well-granulated beta cells were found associated with large numbers of older degranulated beta cells with aldehyde-fuchsin-positive borders. The degranulation and increased mitotic activity of beta cells, as well as the neogenesis of beta cells from duct epithelium with islet hyperplasia, are indicative of increased insulinogenic ability of the pancreas subjected to prolonged hyperglycemia. This capacity to increase insulin output is thought to account for the adaptation of the rabbit to the diabetogenic agents and for the gradual diminution in diabetogenic effectiveness of the hormonal treatment.

Acknowledgment

The microphotographs that illustrate this article were prepared by Herbert A. Fischler.

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STUDIES ON THE MECHANISM OF CAPTURE AND DEGRADATION OF INSULIN-I^{131} BY THE CYCLICALLY PERFUSED RAT LIVER

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Since liver extracts have been shown to contain a potent insulin-degrading activity,[1-3] we were prompted to investigate the fate of insulin in an intact liver preparation designed to preserve as well as possible the architectural integrity of isolated liver tissue and its cells. The rate of insulin degradation thus observed would reflect, not only the activity of the insulin proteolytic system, but also processes that govern the rate of entry of insulin into the cell, assuming an intracellular location for this system. Thus, failure to observe degradation under these conditions, despite the presence of insulinase in liver extracts, would argue strongly against a significant role of the liver in the degradation of circulating insulin. It was found, however, as reported in a previous communication,[4] that iodoinsulin was rapidly degraded by the intact, cyclically perfused rat liver. In the following report we review these preliminary perfusion studies and present the results of more recent experiments designed to explore possible mechanisms of entry of iodoinsulin into the liver cell.

Perfusion Experiments at 37°C.

FIGURE 1 is a schematic drawing of a recent modification of the perfusion apparatus used in many of these experiments.[4] Heparinized whole rat blood was added to a round-bottomed flask reservoir-oxygenator. As the flask was turned about its axis and slanted a few degrees from the horizontal, a film of blood was formed over its lower portion, permitting gas equilibration with an internal atmosphere of humidified 95 per cent oxygen and 5 per cent carbon dioxide. After filtration, the blood was pumped through a portal vein cannula into the liver *in situ*, returning to the flask by means of a tube secured in the inferior vena cava above the diaphragm. Retrograde leakage of blood was prevented by ligating the inferior vena cava between the liver and the right kidney. A flow rate of 7.0 ml./min. was maintained in most of these experiments and was estimated to approximate normal hepatic blood flow for rat livers averaging 8 gm. in weight.[4]

The fate of iodoinsulin* after its addition in small amounts to the reservoir of the system described is depicted in FIGURE 2. The perfusate in the reservoir was sampled at the times indicated and the separated plasma treated with trichloroacetic acid (TCA).[4] The radioactivity thus associated with the plasma-TCA precipitate was considered to be a measure of undegraded iodoinsulin, and the products of iodoinsulin degradation were found in the plasma TCA-soluble fraction. It may be seen in FIGURE 2 that by 60 min. of perfusion 70 per cent of the iodoinsulin initially added was removed from the circulating perfusate. We reported previously that the total quantity of TCA-precipitable radioactivity removed by the liver during the perfusions equaled the sum of the final plasma TCA-soluble radioactivity and the radioactivity accumulated by the cellular fraction of the blood.[4] The latter was assumed to be derived from the products of iodoinsulin degradation, since the percentage of radioactivity found in the cellular fraction of blood with respect to the quantity of radioactivity in the TCA-soluble portion of plasma appeared to be fairly constant throughout the perfusions and was of the order of 60 per cent.[4] Thus, it would seem reasonable to conclude that by 60 min. essentially all of the iodoinsulin that had been captured by the liver was degraded and its products returned to the circulation. Of particular interest was the pronounced lag in the early appearance of TCA-soluble radioactivity when compared with the rapid disappearance of iodoinsulin (FIGURE 2). This discrepancy between the initial rates of iodoinsulin capture and the appearance of its degraded products, as will be shown below, would suggest that iodoinsulin was accumulated in the liver during the early phase of its disappearance, a phenomenon pointing to a separate insulin-capturing mechanism.

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* Obtained from Abbott Laboratories, Oak Ridge, Tenn.

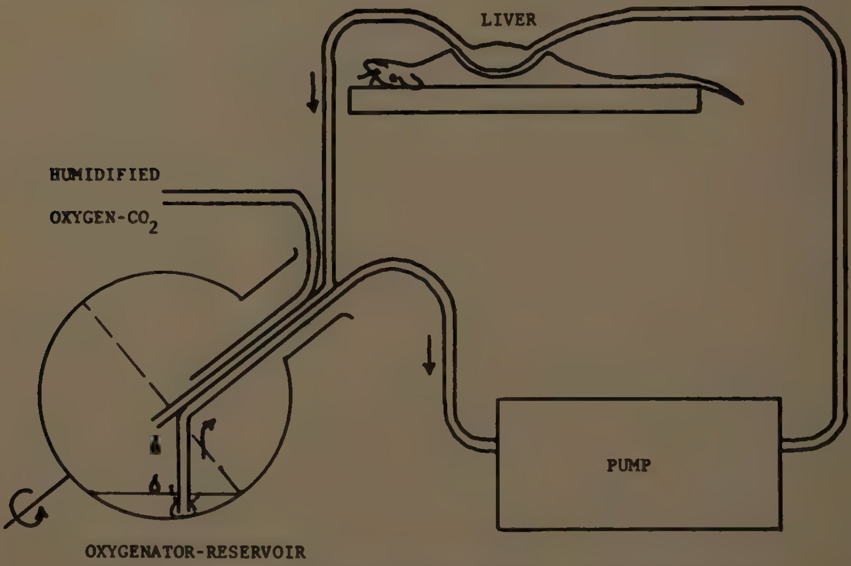

FIGURE 1. Schematic of organ perfusion apparatus. See text for description.

Evidence that at least a major fraction of iodoinsulin is handled in a manner similar to insulin is revealed in the competition that exists between native insulin and its iodinated derivative for one or more limiting steps in the degradative pathway (FIGURE 2). When iodoinsulin was diluted with increasing amounts of native insulin, increasing inhibition in both the rates of disappearance and degradation of iodoinsulin was observed.[4] It would appear, then, that the liver cannot effectively distinguish native insulin from at least a major portion of iodoinsulin.

The 60-min. TCA-precipitable value in FIGURE 2 was shown, in perfusions carried out for extended periods of time, to be very close to the limit of removal.

It was thus estimated that about 25 per cent of the iodoinsulin was relatively unreactive or resistant to capture by the liver. The iodoinsulin used in these experiments was estimated to have a mean iodine content of 1 atom per molecule of insulin (6000 M.W.). At this level of iodination, we have shown previously that the diiodotyrosine (DIT) content is of the order of 15 per cent of the total iodotyrosine residues by weight.[4] The existence of DIT in such an iodoinsulin preparation thus signifies a nonuniform distribution of the iodine label and hence the possibility that some iodoinsulin molecules are more heavily iodinated than others. In order to determine whether this heterogeneity might have contributed to this resistant fraction, the relative DIT content of the perfusate was determined after prolonged perfusion. Plasma samples from the beginning and end of a 120-min. perfusion were dialyzed to remove nonprotein radioactivity and then digested with pancreatin. The digests were chromatographed and the radioactivity associated with MIT and DIT was measured for each sample (FIGURE 3). The significant increase in the proportion of DIT to MIT that became evident after all of the "active" TCA-precipitable radioactivity had been removed strongly suggests that the "inactive" fraction of iodoinsulin is composed of species having a higher than average iodine content. It must be assumed, however, that the reiodination of other protein material had not occurred during the perfusion.

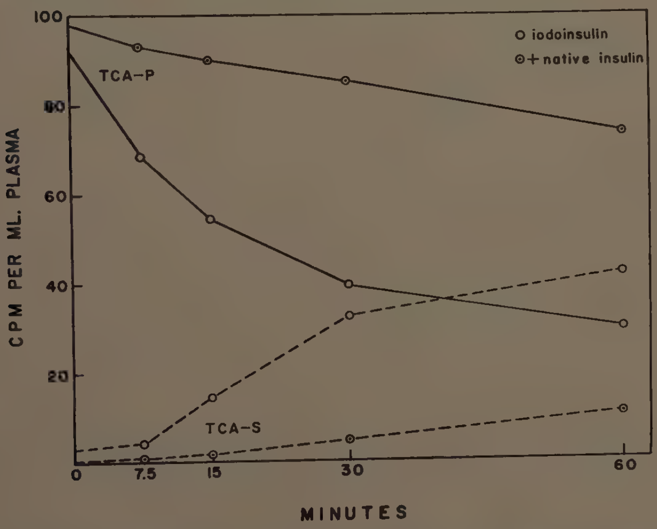

FIGURE 2. Fate of iodoinsulin with and without added native insulin during the cyclic perfusion of isolated rat livers at 37°C. In each experiment, 1.0 μg. of dialyzed iodoinsulin was added to the perfusate at 0 min., yielding an initial concentration of .02 μg./ml. of blood. The plasma TCA-soluble time courses are depicted by broken lines; the plasma TCA-precipitable time courses by solid lines. The open circles (○) are the means of 5 perfusions with iodoinsulin alone. The dotted circles (⊙) represent averages of 2 experiments in which 1000 μg. of crystalline zinc insulin (beef) was added to the iodoinsulin solution.

A calculation of the velocity constant for the hepatic removal of "active" iodoinsulin has been previously reported.[4] For a more complete kinetic discussion the reader is referred to that communication, from which the following considerations were taken. Since the volume of perfusate contained within the liver, tubing, and pump was sufficiently small with respect to its rate of flow, it may be said that the velocity of iodoinsulin uptake by the liver at any time will cause essentially "instantaneous" concentration changes within the reservoir perfusate. The mean plasma TCA-precipitable values for iodoinsulin disappearance shown in FIGURE 2 were corrected by subtracting from each the limiting or asymptotic TCA-precipitable value. From these corrected values a velocity constant of approximately 3 ml./min. was computed. Such a constant, which differs from the traditional constant $k = l/t$, is independent of changes in the volume of perfusate and, within limits, is also independent of the flow through liver. It may therefore be viewed as a quantitative approximation of the kinetics of the reaction occurring within the liver itself.

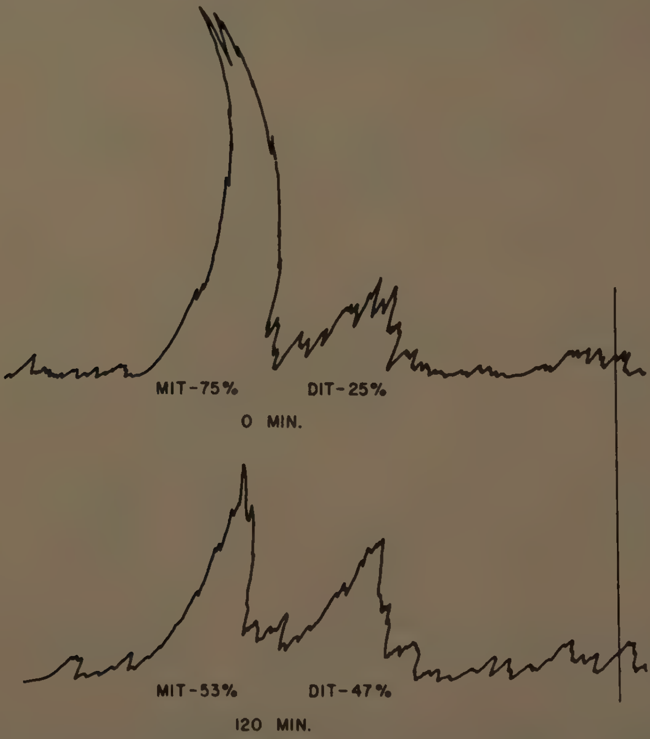

FIGURE 3. Radiochromatograms of enzymatic digests of dialyzed plasma taken initially and after 120 min. of liver perfusion at 37°C. Ten μg. of iodoinsulin was added to the perfusate at 0 min. The vertical line has been drawn through the 2 origins. The areas under each peak were determined planimetrically and indicate the relative amount of radioactivity associated with MIT and DIT.

The percentage of iodoinsulin removed with respect to the amount delivered to the liver at any time, however, is related directly to the flow of perfusate. Having a velocity constant of removal of 3 ml./min. means that, of the iodoinsulin entering the liver, the amount removed will equal that contained in 3 ml. of the perfusate. Thus, since the established flow was 7 ml./min., three sevenths (40 per cent) of "active" iodoinsulin will be taken up during a single liver passage. This percentage extraction under simulated physiological conditions agrees very well with similar estimations made in intact rats.[5]

Cooled-Liver Perfusion Studies

The degradation of iodoinsulin by liver homogenates has been shown to be temperature-dependent, having a Q_{10} from 25° to 40°C. of approximately 2.[1] If the degradation of iodoinsulin within the liver cell involves similar reactions, then one should observe a substantial reduction in degradation by lowering the temperature of perfusion from 37° to nearly 0°C. With this in mind, a series of experiments was conducted in a 3°C. cold room. Livers from rats of the same sex, strain, and weight were perfused as before. The cell mass, however, was removed from the perfusate to reduce viscosity, and the plasma was equilibrated with air by constant stirring in a beaker surrounded with cracked ice. Since oxygen consumption was virtually nil at this temperature, it was thus possible to perfuse livers for periods as long as 4 hours with no visible evidence of swelling or deterioration. Although iodoinsulin disappeared from the cooled perfusate, there was no increase in TCA-soluble radioactivity, either in the plasma or within the liver by the end of the experiment, indicating a complete cessation of iodoinsulin degradation (FIGURE 4). Furthermore, since all the TCA-precipitable radioactivity which was removed could be recovered from the liver as TCA-precipitable material, virtually none was bound to the tubing or the glass reservoir. When a large amount of native insulin was dissolved in the iodoinsulin solution (FIGURE 4) the disappearance of the latter was nearly

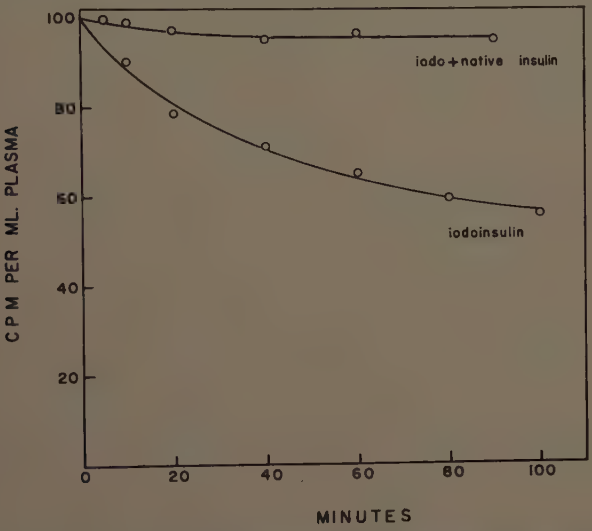

FIGURE 4. Effect of native insulin on the binding of iodoinsulin by the cyclically perfused cooled rat liver. The curve with iodoinsulin alone is the mean of 4 experiments in which 0.1 μg. of dialyzed iodoinsulin was added to 25 ml. of the plasma perfusate at zero time. No iodoinsulin degradation was observed. The upper curve reveals the competition observed with 1000 μg. of crystalline zinc insulin (beef).

abolished, thus establishing the existence of a saturable site of removal and eliminating the possibility that iodoinsulin nonspecifically diffused into fluid spaces of the liver.

The essential irreversibility of this disappearance is indicated in FIGURE 5. As in earlier experiments, iodoinsulin was removed by the liver during 100 min. of perfusion. Immediately thereafter, the plasma in the reservoir was exchanged for fresh plasma. After 5 min. of circulation to permit equilibration, a zero-time sample was taken, leaving a circulating volume equal to the initial volume. Note the barely perceptible increase in TCA-precipitable radioactivity during the second half of this experiment. The implication that the process of removal was one of strong binding to the liver was strengthened by the results of the following experiment. At the termination of a separate 100-min. cold perfusion, residual unbound iodoinsulin was eluted from the liver by perfusing with 0.25 M sucrose. A portion of the liver was then minced and homogenized in an 0.25-M sucrose medium containing native insulin (40 μg./ml.). The concentration of native insulin was such that, should iodoinsulin become unbound during homogenization, it would likely remain unbound. After centrifuging at 100,000 g for 30 min. the supernatant fraction, when compared with the whole homogenate, carried only 11 per cent of the total radioactivity, one half of which was TCA-soluble. Inasmuch as the presence of native insulin should hinder or even reverse binding, the fact that nearly all of the captured iodoinsulin sedimented under these conditions establishes the existence of stable binding between iodoinsulin and a particulate fraction of the liver cell.

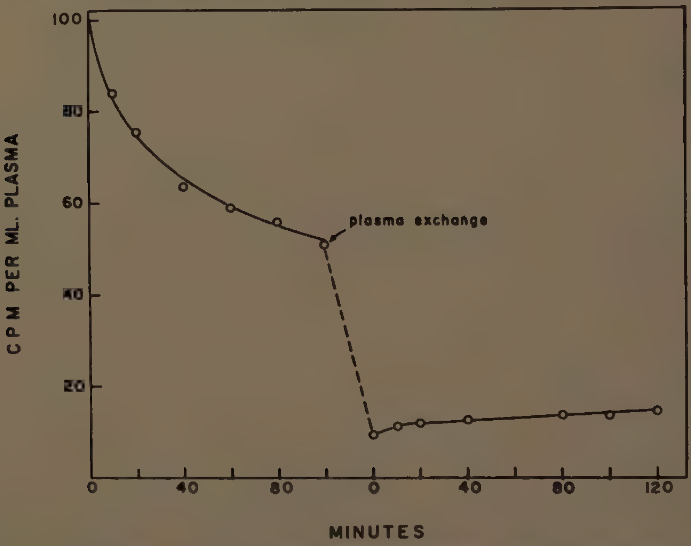

FIGURE 5. An attempt to elute bound iodoinsulin from cooled perfused rat liver. The experimental conditions during the first 100 min. were identical to those described for the lower curve in FIGURE 4. As described in the text, the perfusing plasma was exchanged for fresh plasma (without iodoinsulin), and the cyclic perfusion restarted. During the second phase, the slight rise in radioactivity indicates the essential irreversibility of binding.

Failure to detect any degradation during the cold perfusions was surprising, since one would have predicted on the basis of liver homogenate experiments a sharply reduced, yet distinctly measurable rate of degradation at this temperature. For this reason, the potentiality of the cold liver to degrade bound iodoinsulin at 0°C. was tested. A 100-min. cold perfusion was run as before and, at its conclusion, unbound iodoinsulin was eluted from the liver with saline.

TABLE 1
DEGRADATION OF BOUND INSULIN-I^{131} BY SLICES AND HOMOGENATES OF COOLED, PERFUSED RAT LIVER
Per Cent TCA-Soluble Radioactivity After 1-Hour Incubation

Incubation temperature	Slice	Homogenate
0°C.	3	22.1
9°C.	9.8	50.2
25°C.	23.3	89.2

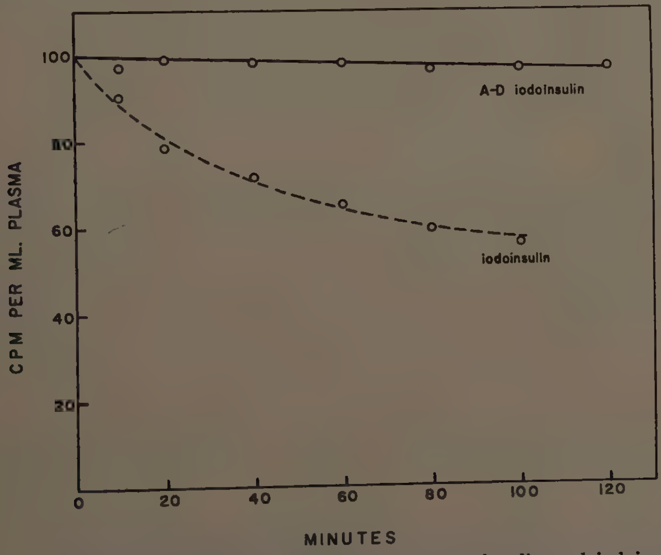

FIGURE 6. Comparative binding of alkali-denatured iodoinsulin and iodoinsulin by the cyclically perfused, cooled rat liver. The initial concentrations of the 2 iodoinsulin preparations were identical.

A segment of the liver was then homogenized in 0.1 M Tris buffer, *p*H 7.6, yielding a 10 per cent homogenate. Portions of this homogenate were incubated at 3 different temperatures, 0°, 9°, and 25°C. Slices of the same liver, moistened with saline, were similarly incubated. After 60 min. the incubations were terminated by the addition of cold TCA, together with homogenization of the slices. The TCA-soluble radioactivity, when compared with the total for each sample, indicated the amount of bound iodoinsulin that was degraded during the incubation; the results are shown in TABLE 1. Note that 22 per cent of the bound iodoinsulin was degraded during the 0°C. homogenate incubation, a rate 7 times greater than that observed with the slice. Similar relationships were seen at higher temperatures.

To explain this complete inhibition of iodoinsulin degradation in the intact liver where proteolytic activity, as noted in the above experiment, was only partially inhibited by cooling, it is reasonable to evoke the idea that an essential element was physically separated from the other components of the degradative system. Such an element might be an enzyme, cofactor, or iodoinsulin itself. If iodoinsulin is the isolated component and it is further assumed that the site of binding is on or within the cell where degradation occurs, then one may postulate the existence of an insulin transport or transfer mechanism, which may be more sensitive to the effects of cooling than is the degradative system itself.

Some degree of selectivity in the binding of iodoinsulin is indicated by its very removal from plasma, a medium abundant in protein. Furthermore, iodoinsulin that was altered by treatment with dilute alkali failed to bind (FIGURE 6). Such treatment is known to abolish the hypoglycemic activity of insulin by changing its secondary structure and possibly disrupting disulfide linkages.[6] Finally, no competition was noted between iodoinsulin and glucagon or prolactin for hepatic binding in the cold when the latter were added to the perfusate in amounts comparable to native insulin as shown in FIGURE 4.

Comments

It will be recalled that during the first 15 min. of perfusion at 37°C. a rapid disappearance of iodoinsulin was observed, while the products of degradation increased only very little (FIGURE 2). It was shown subsequently that this initial rate of iodoinsulin disappearance could be markedly inhibited by adding to the perfusate unlabeled native insulin, an observation that lead to the conclusion that a nonspecific diffusion of iodoinsulin into fluid compartments of the liver contributed little, if at all, to its disappearance. Such a dichotomy in rates obviously results in the hepatic accumulation of iodoinsulin or its products. It has been reported that, after administering iodoinsulin to intact rats, most of the radioactivity in livers removed within 15 min. after injection was precipitable with TCA.[7] Furthermore, the hepatic concentration of this material was several times higher than one would expect from simple diffusion alone.[7] Thus, this initial discrepancy in rates and the concomitant accumulation of TCA-precipitable material very likely signify that the hepatic sequestration of iodoinsulin is initially faster than its rate of hydrolysis by intracellular proteolytic enzymes, and would suggest further the presence of an independent capturing mechanism.

In view of the generally extreme degradative potency of liver homogenates, it is not readily apparent why iodoinsulin would be permitted to accumulate unless its site of capture were remote from the degrading system and its subsequent release from this site relatively slow. Thus, the ultimate rate-limiting step in the hepatic degradation of iodoinsulin may be its transfer from the site of capture to the degradative system.

The results of the cooled-liver perfusion experiments are of interest to this discussion since they outline more graphically the hypothesis just presented. The selective binding of iodoinsulin that was observed during liver perfusions in the cold was not followed by degradation, even after 4 hours of continuous perfusion. In spite of the complete inhibition of degradation observed in the intact liver, the proteolytic system was active since an appreciable fraction of bound iodoinsulin was subsequently degraded at 0°C. after the liver was homogenized (TABLE 1). As mentioned earlier, perhaps an essential component necessary for degradation remained physically isolated in the intact cooled liver, and was brought in contact with the system during homogenization. In line with this reasoning, it is not inconceivable that cooling had completely blocked a thermosensitive transfer step, which was interposed between the binding of iodoinsulin and its degradation. The thermosensitive step in this scheme might then be the rate-limiting transfer process postulated for the 37°C. perfusions.

Summary

Iodoinsulin was shown to be rapidly degraded by the isolated, cyclically perfused rat liver. At a rate of blood flow that was estimated to approximate hepatic blood flow in intact rats, it was estimated that about 40 per cent of the iodoinsulin entering the liver at any time was removed in a single transhepatic circulation when the concentration of iodoinsulin was low.

In an attempt to study the mechanism of iodoinsulin capture it was found that cooling the intact liver and perfusate to 3°C. completely abolished iodoinsulin degradation. Iodoinsulin, however, was removed from the perfusate under these conditions and was subsequently observed to be tightly bound to an unidentified sedimentable fraction of the hepatic cell. Since cooling only partially inhibited hepatic proteolytic activity, the total cessation of degradation that was noted in the cold perfusions suggested that an intermediate thermosensitive step, interposed between the cellular sites of iodoinsulin binding and degradation, was blocked.

Acknowledgments

We thank Otto Behrens of Eli Lilly and Company, Indianapolis, Ind., for supplying the crystalline insulin used in these studies and Robert Bates of the National Institute of Arthritis and Metabolic Diseases for his gift of prolactin.

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RECENT STUDIES ON INSULIN-BINDING ANTIBODIES

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Insulin-binding antibodies uniformly appear in the sera of human subjects after 1 to 3 months of continuous insulin therapy.[1] Since the antigen-antibody complexes are not precipitable and the maximum insulin-binding capacity is generally only of the order of 200 to 300 mμg./ml. serum, detection of the antibody requires special techniques. In our experience, the use of I^{131}-labeled insulin and paper chromatography[1] or electrophoresis[1, 2] has proved most expedient for quantitative as well as qualitative analysis of insulin-antiserum mixtures. When present in low concentrations, free insulin-I^{131} adsorbs firmly to Whatman 3 MM paper at the site of application ("origin"),[1, 3] whereas insulin-I^{131} bound to antibody moves away from the origin during chromatography or electrophoresis[1] (FIGURE 1). The binding of insulin to antibody is also demonstrated by starch-block electrophoresis,[1] cellulose-column chromatography,[4] and preparatory ultracentrifugation of insulin-I^{131}-antiserum mixtures,[1] by precipitation of the insulin-I^{131}-antibody complexes with specific rabbit antihuman globulin serum,[5] and by hemolysis of insulin-sensitized red cells.[6] The insulin-binding antibody in man is not strictly a γ-globulin, but has rather a mobility intermediate between the γ- and β-globulins.[7] The presence of antibody is responsible for delaying the disappearance of insulin from the blood stream[1, 8] and for the protection of insulin against the degradative action of liver insulinase[9] and, probably, of other insulin-degrading enzymes, as well. When present in high concentrations, the insulin-binding antibody is associated with manifest clinical insulin resistance. Whereas the insulin-binding capacities of sera from most insulin-treated patients do not generally exceed 10 U./l. serum,[1, 10-13] the sera of insulin-resistant patients bind from 60 to 500 U./l. and more.[10-14] However, the effect of antibody on insulin requirements depends on a number of other factors in addition to the concentration of antibody. These factors include the rates of formation and dissociation of insulin-antibody complexes, which are in part determined by the energies of reaction between insulin and antibodies and the rate of immunological elimination of complexes.[13] The reversibility of insulin-antibody complex formation[1, 9-13] may result in delayed insulin reactions even several days after large amounts of insulin have been administered during the treatment of insulin resistance associated with high antibody concentrations.[11]

At least two types of insulin-antibody complexes are formed; their rates of dissociation differ by a factor of 25 to 100.[10-13] The standard free-energy changes involved in formation of these complexes (minus 10 to 11 kcal./mol. for one and minus 13 to 14 kcal./mol. for the other[13, 15]) are considerably greater than those reported for most other antigen-antibody reactions.[16] The positive entropy changes in these reactions ($\Delta S^0 = 25–30$ entropy units/mol.)[13] have been interpreted in other antigen-antibody systems as being due to the release of bound water molecules at the sites of reaction.[17]

In general, equilibrium constants were found to be significantly greater in

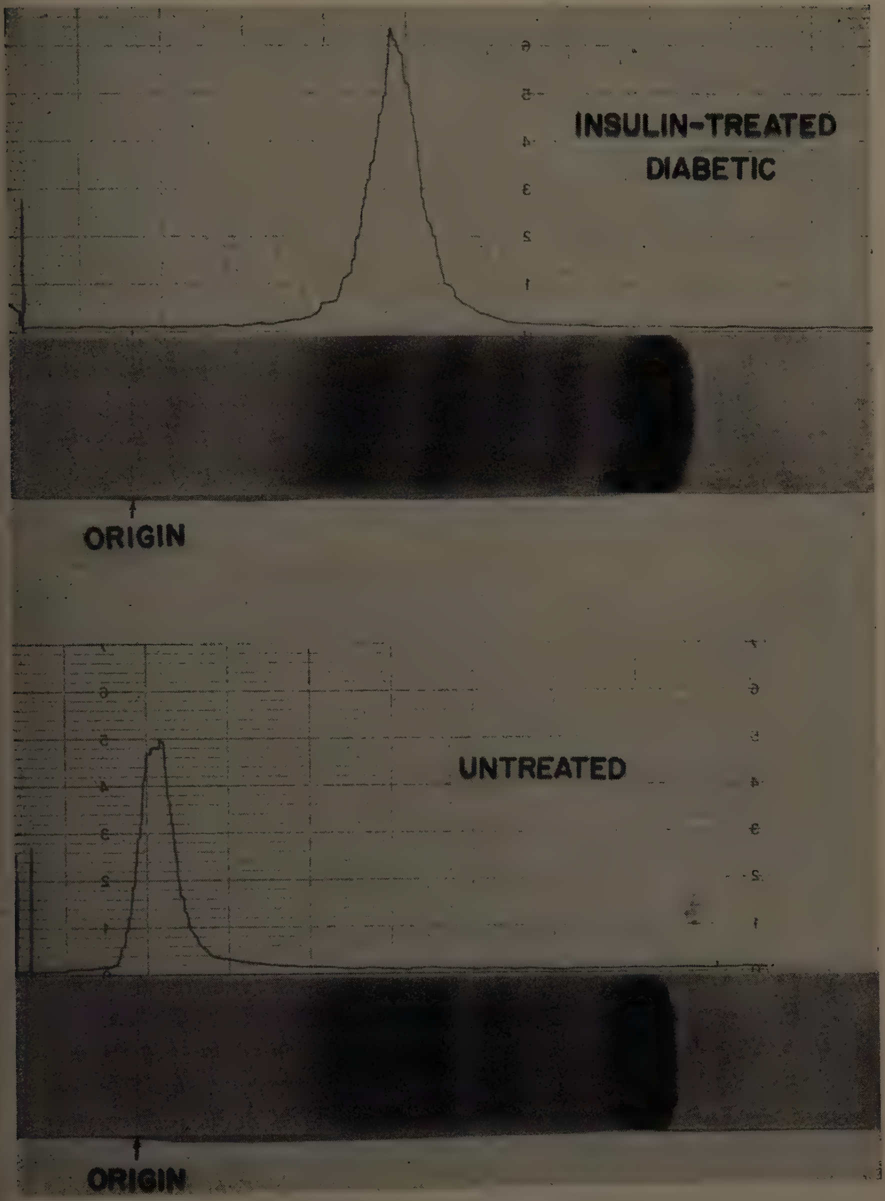

FIGURE 1. Paper electrophoretograms of insulin-I^{131} in the serum of an insulin-treated diabetic subject (*top*) and in the plasma of an untreated control subject (*bottom*), together with radioactive scans of the paper strips.

sera from nonresistant subjects (low antibody concentrations) than from insulin-resistant patients (high antibody concentrations), which would appear to indicate that some loss in specific affinity of antibody for antigens results when antibody synthesis is markedly accelerated in this system.[13]

Recent studies have shown that some antisera manifest marked differences in the energy of reaction with insulins of different animal species. Beef and sheep insulins are generally bound much more strongly than pork and horse insulins,[18, 19] as evidenced not only by greater direct binding of the labeled insulins but also by more effective competitive inhibition on cross reaction (FIGURES 2 and 3). Inasmuch as these various animal insulins differ in amino acid sequence only in the 8, 9, and 10 residues of the A chains,[20] it would seem that this region not only plays an important part in the reaction with antibody but also represents a site of antigenicity. It should not escape attention that the similarly reacting animal insulins contain the same amino acids in the 8 and 10 positions[20] (alanine and valine, respectively, in beef and sheep insulins, and threonine and isoleucine, respectively, in pork and horse insulins). Since most commercial preparations are mixtures of beef and pork insulin, it is of special interest that antisera react more strongly with sheep insulin than with pork insulin, and that the *R* portions of the 8, 9, 10 residues (alanine, glycine, and valine) of sheep insulin *A* chain contain no permanently charged groups.

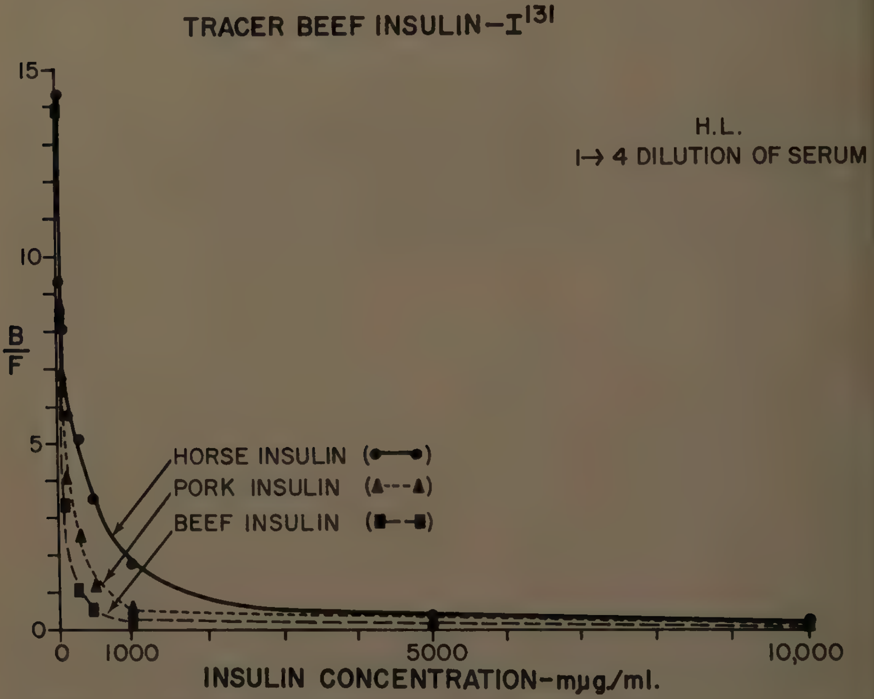

FIGURE 2. Ratio of bound to free beef insulin-I^{131} (tracer concentrations) as a function of concentration of unlabeled horse, pork, and beef insulins.

In the light of this fact, the high energy of interaction of sheep insulin with antibody has been interpreted[19] as indicating the important contribution of van der Waals' attractive forces (in consequence of close spatial complementarity of antibody and antigen) to the stability of the antigen-antibody complex. In support of this conclusion is the observation that the equilibrium constants are not significantly dependent on the ionic strength of the solutions within wide limits of the latter.[4]

The specific quantitative relationship between the concentration of insulin present in insulin-antiserum mixtures and the percentage of insulin-I^{131} that becomes bound to antibody has been used to advantage in the development of an immunoassay for insulin.[21] However, the assay is necessarily specific for individual animal insulins and not for the hormonal activity of insulin and therefore is not affected by the presence of other insulin antagonists. Thus, this method can assay the concentration of any insulin (for example, beef, pork, or human), provided that the characteristics of the reaction of the particular insulin of interest with a particular antiserum are established. It is not possible by these means to assay insulins for which no standard preparation is available but, if the insulin content is known, even a crude preparation will suffice. The method is briefly as follows:

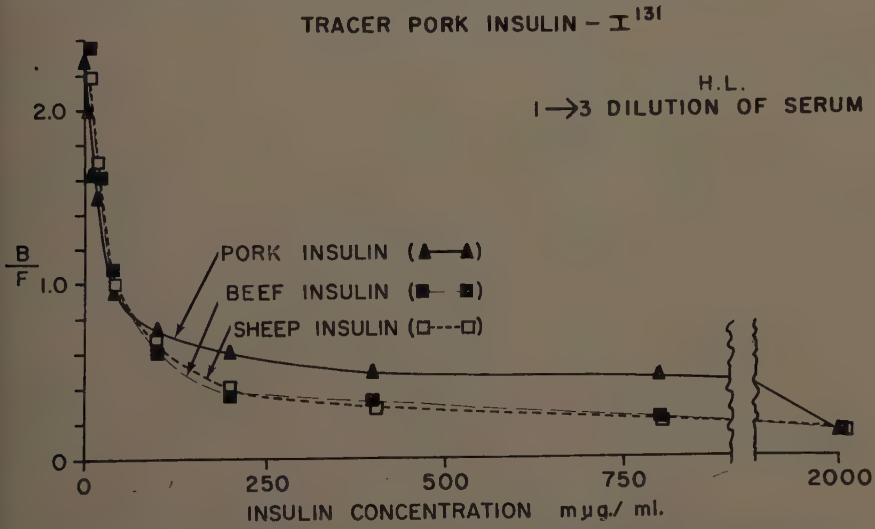

FIGURE 3. Ratio of bound to free pork insulin-I^{131} (tracer concentrations) as a function of concentration of unlabeled pork, beef, and sheep insulins. Note the lower initial B/F ratio for pork insulin-I^{131} than for beef insulin-I^{131} (FIGURE 2) in spite of the greater dilution of anti-serum in the latter case.

A series of insulin solutions of known concentration is prepared, and to each solution is added tracer insulin-I^{131} in amounts that are small compared to the quantity of unlabeled insulin present. The same amount of tracer insulin-I^{131} is added also to unknown samples. Following incubation of all solutions with the same concentration of antiserum, the mixtures are analyzed for their content of bound insulin-I^{131} (B) and free insulin-I^{131} (F). The ratio B/F is then plotted as a function of the insulin concentration present in the known solutions to provide a "standard curve." By reference to the standard curve the insulin concentration in unknown samples is readily determined from the observed B/F values. If the unknown solutions are assayed at several different concentrations, the B/F values fall along different regions of the standard curve and the precision of the determinations is thereby increased. The methods and results obtained in the assay of a crude extract of beef pancreas are illustrated in FIGURES 4 and 5. The values obtained were in good agreement with the results of assays by the mouse convulsion test.[21] * Since the slope of the curve of B/F versus insulin concentration decreases with increase in insulin concentration, the change in B/F for any given percentage increment in insulin will be greater as the initial insulin concentration is lower. Furthermore, since the equilibrium constants of the reactions between insulin and human antibodies increase twofold at 4°C. compared to 37°C.,[13] the precision and the sensitivity of the assay are approximately doubled if the mixtures are allowed to remain in the refrigerator for 1 or 2 days after incubation at 37°C. for several hours.

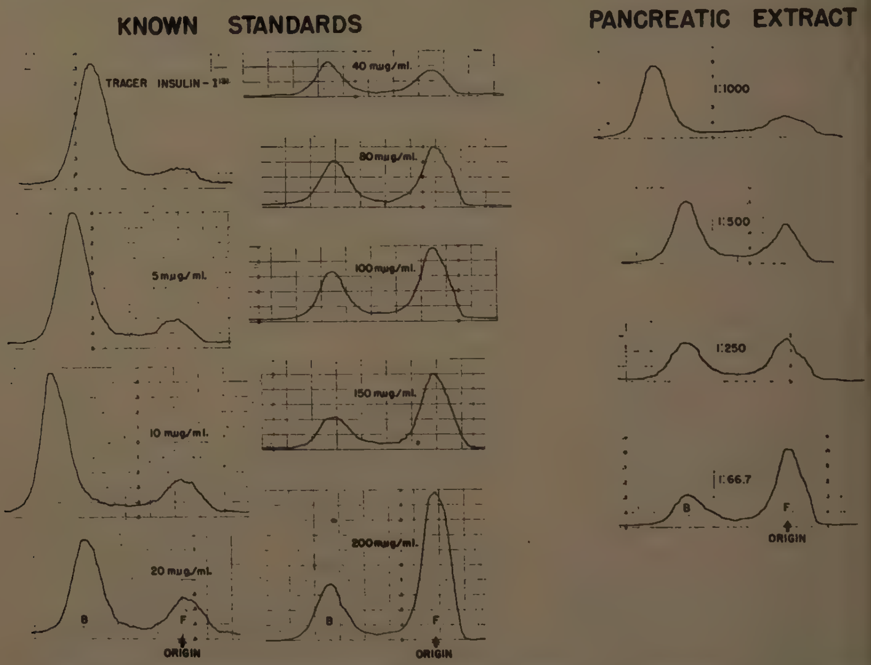

FIGURE 4. Paper radiochromatograms of beef insulin-I131 in antiserum with various known concentrations of unlabeled beef insulin (*left*) and with various dilutions of a crude extract of beef pancreas (*right*). The pancreatic extract was supplied by Reuben Schucher of the Montreal Jewish Hospital.

Selected human antisera permit the detection of beef insulin concentrations as low as 5 or 10 microunits/ml., but any given reduction in B/F ratio for beef (or pork, or horse, or sheep) insulin-I^{131} requires from 25 to 100 times as much human as animal insulin. Therefore, human antisera produced against beef or pork insulins are not very satisfactory for assay of human insulin at the concentrations that usually obtain in blood. However, human insulin has been found to react well with guinea pig antisera to beef insulin,[4] so that the lack of availability of adequate amounts of human insulin for immunization purposes is not too distressing. Because human insulin is not readily available in quantities sufficient for standardization of different antisera in many laboratories, we are attempting to stockpile a large quantity of a specific guinea pig antiserum that is to be accurately standardized with beef insulin-I^{131} and human insulin. We may then be in a position to supply interested investigators with such standardized antisera, together with the appropriate calibration curves. The study of endogenous insulin concentrations in man may thus be expedited. The problem is to obtain an antiserum with a binding capacity sufficiently high to permit use of the antiserum at high dilution such as 1:100 or 1:200, and with a high equilibrium constant so that it will be sensitive to the low insulin concentrations present in the blood stream. From present indications it appears that this will be quite feasible, but several months will elapse before standardized sera will be available. Another difficulty that may

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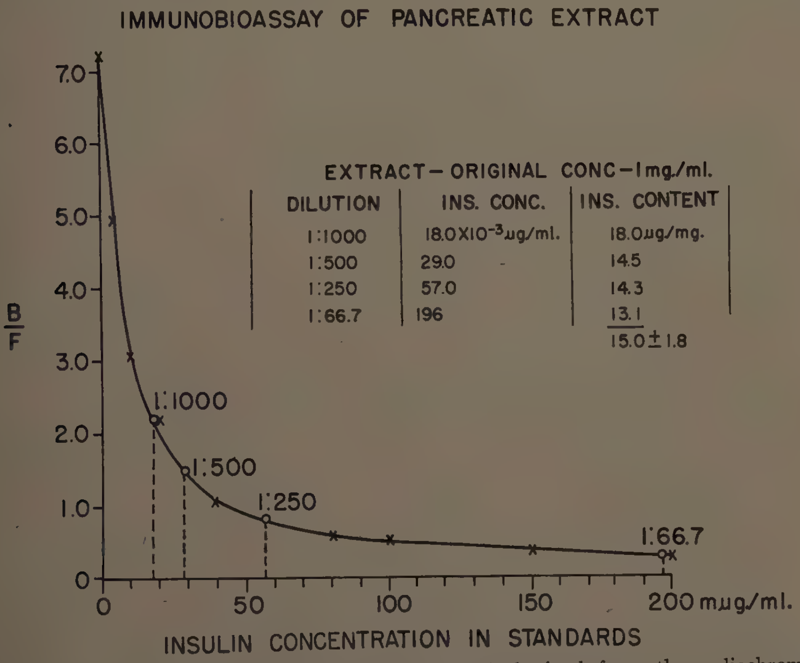
* The mouse convulsions assays were performed by Reuben Schucher of the Montreal Jewish Hospital, Montreal, Que., Canada.



EXTRACT—ORIGINAL CONC—1 mg./ml.

DILUTION	INS. CONC.	INS. CONTENT
1:1000	18.0×10⁻³μg/ml.	18.0μg/mg.
1:500	29.0	14.5
1:250	57.0	14.3
1:66.7	196	13.1
		15.0 ± 1.8

FIGURE 5. Ratios of bound to free beef insulin-I^{131} obtained from the radiochromatograms of FIGURE 4, are plotted as a function of insulin concentration in known insulin standards (×). The *B/F* ratios in the various dilutions of beef pancreatic extract (○) are plotted along the standard curve.

be mentioned only briefly here is that of producing the required high-specific-activity insulin-I^{131} free of excessive amounts of irradiation-damaged components.[1, 22] However, to a large extent this problem has been solved by judicious attention to procedures that limit such damage[23] and in any event can be circumvented by simultaneously running the mixtures with control sera to correct for nonspecific plasma protein-binding of damaged insulin-I^{131}.[13]

Acknowledgments

We are indebted to O. K. Behrens, C. W. Pettinga, H. Neurath, F. Tietze, J. Field, and L. C. Craig for gifts of the various insulins used in these studies.

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INSULIN-STIMULATED GLYCOGEN FORMATION IN RAT DIAPHRAGM*

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Introduction

Insulin-stimulated glucose uptake and glycogen synthesis in isolated rat diaphragm was originally demonstrated by Gemmill and Hamman.[1] Brown *et al.*[2] have reported that, following a 30-min. incubation period, about 75 per cent of the increased glucose uptake in the presence of insulin could be accounted for as tissue glycogen. In the present report it is shown that, after a 10-min. incubation period at 38°C., more than 90 per cent of the increased glucose uptake in the presence of insulin could be accounted for as tissue glycogen, the major increase being in a perchloric acid-soluble glycogen fraction.

Analyses of tissue acid-soluble phosphate intermediates, including inorganic phosphate, creatine phosphate, adenosine diphosphate (ADP), and adenosine triphosphate (ATP) showed no difference between control and insulin-treated diaphragms. A small but statistically significant increase in hexose-6-phosphate content of the insulin-stimulated diaphragms was observed at 2 medium glucose levels.

Glycogen synthesis occurred with ratios of inorganic phosphate to glucose-1-phosphate that would markedly favor glycogen degradation by phosphorylase as studied in soluble systems. Enzyme studies in broken-cell preparations indicate the presence in skeletal muscle of 2 enzymes, uridine diphosphate glucose (UDPG) pyrophosphorylase,[3] and UDPG glycogen transglucosylase,[4] constituting a reaction sequence (independent of inorganic phosphate) converting glucose-1-phosphate to glycogen. These enzymes are of sufficient activity to account for the insulin-stimulated glycogen formation observed.[5]

Materials and Methods

Twelve male Wistar rats weighing between 70 and 100 gm. after an 18 to 24-hour fast were used for each experiment. Incubations were carried out in Gey and Gey bicarbonate buffer[6] and gassed with 95 per cent O_2 and 5 per cent CO_2. Vessels were shaken at 140 cycles per minute in the metabolic shaker.

Prior to the 10-min. experimental period at 38°C., 2 pre-experimental procedures were used routinely: first, a 5-min. preincubation period at 38°C. with shaking and gassing in medium containing no substrate and, second, a 20-min. period at 0°C. without shaking or gassing in medium containing substrate. The first step was designed to lower the endogenous insulin content; the second, to allow insulin fixation to muscle cells to occur. Following the second period, the experiment was run for 10 min. at 38°C. Amorphous insulin (0.1 U./ml.)

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* The work reported in this paper was supported in part by grants from the Cleveland Diabetic Fund, Cleveland, Ohio, the Fund for the Study of Diabetes and Related Metabolic Disorders, and from the National Institute of Allergy and Metabolic Disease (Grant A-2366), Public Health Service, Bethesda, Md.

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was freshly prepared each day prior to use. Following the experimental period, beakers were rapidly chilled at 0°C.; then the tissue was removed, quickly washed, blotted, and frozen in solid-liquid isopentane prechilled in liquid nitrogen. Medium samples were deproteinized by the Ba-Zn method for glucose analysis. The experiment was carried through with pairs of animals according to a timed schedule.

Tissue Extraction

Twelve control and 12 insulin-treated hemidiaphragms from paired vessels were pooled and mechanically reduced to a fine powder in a fitted stainless steel homogenizer chilled in liquid nitrogen. From 500 to 700 mg. of the powder was transferred rapidly to a tared homogenizer tube containing cold 3 per cent perchloric acid. Tissue was weighed and extracted twice at 0°C. by homogenizing for 5 min. with a mechanically driven plastic (Teflon) pestle. Extracts were combined, brought to a known volume, and neutralized by adding solid $KHCO_3$ to neutral *p*H, $KClO_4$ was removed by centrifugation, and the supernatant fluid was used for the various analytical techniques employed.

Analytical

Medium glucose uptake was determined as reducing power by the method of Nelson.[7] Acid-labile phosphate was determined after a 30-min. hydrolysis in NH_2SO_4 at 100°C. Creatine phosphate was estimated by the method of Lowry and Lopez[8] with acetate buffer at *p*H 5.0. Anthrone analysis was done in a final volume of 3 ml. with a concentration of H_2SO_4 of 66 per cent.

Hexose phosphates were estimated microenzymatically in lyophilized neutralized perchlorate extracts of diaphragm or in lyophilized Dowex-2 column fractions. For each determination, a major portion of the perchlorate extract was disolved in a small aliquot of water. Glucose-6-phosphate dehydrogenase and crystalline phosphoglucomutase in the presence of added glucose-1, 6-diphosphate were used. Since the glucose-6-phosphate dehydrogenase preparation is contaminated by isomerase, no distinction was made between glucose-6-phosphate and fructose-6-phosphate. Control experiments established the absence of hexokinase and phosphoglucomutase in the glucose-6-phosphate dehydrogenase preparation.[9] Prior to each experiment, enzymes were tested for the recovery of added glucose-1-phosphate. Recovery was also tested by occasional addition at the end of the determination.

Residual glycogen was estimated after digestion of insoluble diaphragm residue with 1 to 2 ml. of 10 per cent KOH at 100°C. for 60 to 90 min. Glycogen was precipitated with 66 per cent ethanol. Precipitates were washed twice with 66 per cent ethanol before dissolving in a known volume of water.

Perchloric acid-soluble glycogen was estimated by subtracting the total carbohydrate content (anthrone) of the neutralized from the unneutralized perchloric acid extracts. This simple method depends on the trapping of glycogen on $KClO_4$, and has been found to be a reliable and convenient method of estimation of acid-soluble glycogen.

Results

Glucose uptake and glycogen synthesis. Balance studies relating insulin-stimulated glucose uptake to glycogen synthesis following the 10-min. incubation at 38°C. are presented in TABLE 1. Glucose uptake values are reported in the units mg./gm./10 min. When expressed in the more conventional units mg./gm./hr., the values range from 8.6 to 14.5. These values are about twofold higher than values reported for diaphragms from rats of similar size incubated for 30 min.[10] The higher values are probably close to initial rates, since rates decrease with prolonged incubation.[2]

More than 90 per cent of the increased glucose uptake in the presence of insulin could be accounted for as increased tissue glycogen at medium glucose concentrations of 140 and 280 mg./100 ml. At both concentrations the major increase noted was in the acid-soluble glycogen fraction.[11] At 280 mg./100 ml. medium glucose, a statistically significant increase was noted in the residual glycogen fraction. Work is in progress to characterize these 2 operationally separable fractions. These experiments indicate that, after short incubation periods on a balance basis, the increased glucose uptake in the presence of insulin in this preparation can be accounted for to an almost quantitative extent as increased tissue glycogen.

TABLE 1

MEDIUM GLUCOSE UPTAKE AND GLYCOGEN FORMATION BY CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

(Medium Glucose Uptake)

Medium glucose concentration mg./100 ml.	Control mg./gm./10 min.	Insulin mg./gm./10 min.	Mean difference mg./gm./10 min.
140	1.43 (13)*	1.82 (13)	0.39 ± 0.027 <0.001†
280	1.95 (13)	2.41 (13)	0.46 ± 0.049 <0.001†

Tissue Glycogen Content

Perchloric acid-soluble			Residual			
Control mg./gm.	Insulin mg./gm.	Mean difference mg./gm./10 min.	Control mg./gm.	Insulin mg./gm.	Mean difference mg./gm./10 min.	Glycogen yield per-centages
0.814 (10)	1.172 (10)	0.358 ±0.053 <0.001†	1.641 (9)	1.687 (9)	0.046 ±0.034 <0.25 >0.20†	92
0.774 (8)	1.073 (8)	0.299 ±0.062 <0.01†	1.438 (9)	1.560 (9)	0.122 ±0.038 <0.02 >0.01†	92

* Number of experiments indicated by the figure in parenthesis. Each experiment is the mean of 6 control and 6 insulin-treated vessels, each vessel containing 2 hemidiaphragms.

† p.

Acid-soluble phosphates. Analyses of acid-soluble phosphate classes are presented in TABLE 2. These analyses were done on neutralized perchlorate filtrates from diaphragms incubated at a medium glucose concentration of 140 mg./100 ml. Acid-labile phosphate represents 37.9 to 41.4 per cent of the total acid-soluble phosphate. These values are in agreement with values reported for heart,[12] using a rapid-freezing technique for tissue fixation. No significant differences were noted in inorganic phosphate or creatine phosphate. With an insulin-stimulated glycogen synthesis of 2 μmoles/gram/10 mins. no decrease in acid-labile phosphate was noted; on the contrary, a small but statistically significant increase was observed.

ADP and ATP. That this increase was not due to an increase in ADP or ATP was shown by analysis of these compounds after separation on Dowex-2 chloride ion exchange resins. Analyses were carried out by 2 independent measurements: namely, ultraviolet absorption at 260 μ, and radioactivity, using P^{32}. For these experiments animals were preinjected with P^{32}-inorganic phosphate (2 mc. per rat) 18 to 24 hours prior to the experiment.

TABLE 2

ACID-SOLUBLE PHOSPHATE ANALYSIS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

	Inorganic phosphate		Phosphocreatine		Acid-labile phosphate	
	Control μmoles/gm.	Insulin μmoles/gm.	Control μmoles/gm.	Insulin μmoles/gm.	Control μmoles/gm.	Insulin μmoles/gm.
	7.36	9.71	5.02	5.07	7.73	8.07
	8.60	8.06	5.22	6.14	10.40	11.80
	9.87	7.46			9.87	10.50
	9.76	9.82	3.76	2.42	7.05	8.66
	8.43	9.12	4.74	4.63	8.35	9.10
	6.91	7.10	5.00	3.87	7.75	7.76
Mean.....	8.49	8.55	4.75	4.43	8.53	9.32
Mean difference					0.79 ± 0.25	
p.........	0.5		<0.5 >0.4		0.025	

TABLE 3

ADP AND ATP CONTENTS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

ADP (as adenine)		ATP (as adenine)	
Control μmoles/gm.	Insulin μmoles/gm.	Control μmoles/gm.	Insulin μmoles/gm.
1.49	1.31	2.39	2.81
0.68	0.85	1.64	1.65
0.75	0.78	1.74	1.56
0.93	0.87	2.25	2.36
Mean: 0.96	0.95	2.00	2.09

TABLE 3 presents the ultraviolet analytical data for ADP and ATP from such experiments. As may be noted, ADP and ATP levels did not differ significantly between control and insulin-treated diaphragms. Rye Alertson *et al.*[13] have recently reported similar findings after 30-min. incubation periods.

These findings are in agreement with the increased turnover of high-energy phosphate bonds of creatine phosphate and ATP observed in insulin-stimulated diaphragm by Sacks.[14] Calculations show that, in order completely to resynthesize the ATP required for the insulin-stimulated glycogen formation, 6 per cent of the increased glucose uptake would be required, assuming an average P/O ratio of 3 and a requirement of 2 ATPs per mole of glucose polymerized through the UDPG pathway.

Such a coupling of glycogen synthesis to regeneration of ATP could occur through the participation of ADP acting as a specific acceptor. As has been shown, oxidative phosphorylation can be made dependent in an almost absolute sense on the availability of ADP as phosphate acceptor for ATP resynthesis.[15]

Hexose phosphates. Hexose-6-phosphate and glucose-1-phosphate contents of control and insulin-treated diaphragms after 10-min. incubations at 38°C. have been measured. At a medium glucose concentration of 140 mg./100 ml., mean hexose-6-phosphate contents were 0.109 and 0.136 μmole/gm. for control and for insulin-treated diaphragms, respectively (TABLE 4). The small increase noted (0.027 ± 0.008) was statistically significant; mean glucose-1-phosphate contents were 0.034 and 0.028 μmole/gm., respectively. Glucose-1-phosphate levels are considerably lower than hexose-6-phosphate, and in the direction of phosphoglucomutase equilibrium. There was no significant difference between control and insulin-treated tissue contents of glucose-1-phosphate.

TABLE 4

HEXOSE MONOPHOSPHATE CONTENTS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

Medium glucose concentration mg./100 ml.	Glucose-1-phosphate			Hexose-6-phosphate			
	Control μmoles/gm.	Insulin μmoles/gm.	Difference μmoles/gm.	Control μmoles/gm.	Insulin μmoles/gm.	Mean difference μmoles/gm.	p
140	0.034 (6)	0.028 (6)	—	0.109 (7)	0.136 (7)	0.027 ±0.008	<0.02 >0.01
280	0.048 (8)	0.050 (8)	—	0.169 (8)	0.212 (8)	0.043 ±0.014	0.02

Ratio P/G 1 → P (8.49/0.028) = 250 (8.55/0.028) = 305.

Similar findings have been made at a medium glucose concentration of 280 mg./100 ml. Mean hexose-6-phosphate contents were 0.169 and 0.212 μmole/gm. for control and insulin-treated tissue, respectively. The small difference observed (0.043 ± 0.014) was again statistically significant. Mean glucose-1-phosphate contents were 0.048 and 0.050 μmole/gm., respectively, no significant difference being observed in this fraction.

The increase noted in the hexose-6-phosphate fraction of the insulin-treated diaphragm at the 2 medium glucose concentrations is compatible with an insulin-stimulated conversion of medium glucose to tissue hexose-6-phosphate.

The absence of an increase in the glucose-1-phosphate fraction that might be expected to increase concomitantly with the hexose-6-phosphate fraction may be explained in one of two possible ways:

(1) The expected increase is counterbalanced by removal of glucose-1-phos-

phate (presumably to glycogen) through a kinetically rapid reaction, possibly such as the UDPG pyrophosphorylase reaction (see below).

(2) A specific insulin-stimulated removal of glucose-1-phosphate that would lead to the concept of a double effect, but not necessarily double action of insulin in diaphragm.

Ratios of inorganic phosphate to glucose-1-phosphate. In view of the low levels of glucose-1-phosphate observed under these conditions, it was of interest to express these levels in terms of ratios of inorganic phosphate to glucose-1-phosphate. Through the extensive work of Cori[16] and of Hanes[17] it is well recognized that whether synthesis or degradation of glycogen by phosphorylase will occur depends on the value of this ratio, which at equilibrium has a value of 3 at neutral *p*H. Ratios higher than 3 result in glycogen degradation, while ratios lower than 3 result in glycogen synthesis. The concentrations of reactive sites (outer chains) of the glycogen primer do not ordinarily enter into the expression, since their size and not their number changes with lengthening or shortening of the outer chains. The ratios observed in these experiments at 140 mg./100 ml. medium glucose were 250 and 305, respectively, for control and insulin-treated diaphragms (TABLE 4). Such ratios observed in tissue rapidly synthesizing glycogen are 75 to 100 times removed from phosphorylase equilibrium in the direction of glycogen degradation.

Either phosphorylase was catalyzing the synthesis of glycogen under unusual local conditions at the synthesis site, or the synthesis was being catalyzed by another set of reactions independent of inorganic phosphate, an alternate mechanism. Another reason for considering the latter possibility was brought out by the work of Sutherland,[18] who has shown that activation of phosphorylase in either liver or muscle by epinephrine or glucagon is associated with glycogen breakdown.

Enzymes of the uridine-linked pathway of glycogen synthesis in muscle. Our efforts in seeking an alternate pathway were considerably strengthened by the report of Leloir and Cardini.[4] These workers reported the direct polymerization of UDPG to glycogen without the intervention of glucose-1-phosphate as intermediate by an enzyme present in liver. We looked for this enzyme in muscle extracts and were able to identify this activity in a particulate fraction of extracts obtained by homogenizing muscle in hypotonic Tris-Versene; such determinations are shown in TABLE 5. Activity was estimated by two independent methods: (1) net glycogen synthesis and (2) spectrophotometrically by UDP formation. The agreement between those two methods may be fortuitous, since they were done under different conditions. The net-synthesis experiments were done with concentrations of UDPG of 20 μm./ml., with net conversions of the order of 20 per cent of the UDPG to glycogen in a 60-min. incubation period. Recently we have found that the supernatant fluid also contains the enzyme, so that these estimates of activity are probably somewhat low. The spectrophotometric method was run with a concentration of UDPG of 4×10^{-4}M, and initial rates were measured. Rates as measured are of sufficient activity to account for the insulin-stimulated glycogen synthesis observed in these experiments. This enzyme has recently been obtained in soluble form and partially purified. It is of interest in terms of possible control mechanisms that, in confirmation of unpublished findings of Leloir and his co-workers, the glycogen-synthesizing enzyme shows an almost absolute dependence on small amounts of added glucose-6-phosphate (TABLE 6). The amounts required for maximal activation are of the range of hexose-6-phosphate that are present in diaphragm.

UDPG pyrophosphorylase has been measured spectrophotometrically by the pyrophosphate-dependent formation of glucose-1-phosphate. The enzyme is present in the soluble fraction of muscle extracts obtained after centrifugation at 100,000 g for 60 min. Its activity is about ten- to twentyfold higher than the glycogen transglucosylase (TABLE 7) and has a widespread distribution in animal tissues. The combination of these two activities is a reaction sequence leading to glycogen synthesis from glucose-1-phosphate independently of inorganic phosphate.

TABLE 5

UDPG GLYCOGEN TRANSGLUCOSYLASE ACTIVITY OF RAT MUSCLE EXTRACTS

Chemical* mg./100 gm./hr.	Spectrophotometric† mg./100 gm./hr.
576	610
486	783
210	334
986	465
240	527
Mean..............500 (2.78 mmoles)	543 (3.02 mmoles)

* Reaction mixture contained: muscle glycogen, 0.67 μmole; UDPG, 2 μmoles; enzyme, 0.01–0.02 ml. of particles prepared by centrifugation of 1:3 muscle extract at highest speed of Servall 15 minutes and resuspending particles in original volume of hypotonic Tris-Versene; volume 0.1 ml.; temperature 37°C., 60 min.

† Reaction mixture contained (final concentration): phosphoenolpyruvate, 5×10^{-4} M; DPNH, 2×10^{-4} M; Mg^{++}, 2×10^{-2} M; UDPG, 4×10^{-4} M; glycogen, 1×10^{-3} M; glycylglycine buffer, 0.025 M, *p*H 7.4; 0.1 ml. 1–100 dilution of crystalline lactic dehydrogenase (Worthington); enzyme, 0.01 ml. particles; volume, 1 ml.

TABLE 6

EFFECT OF GLUCOSE-6-PHOSPHATE ON UDPG-GLYCOGEN TRANSGLUCOSYLASE ACTIVITY

	γ/Mg. protein/hour
No glucose-6-phosphate added..............................	4.05
5×10^{-4} M glucose-6-phosphate..............................	91.30

Discussion

The presence of these two enzymes opens the question of the enzymatic mechanism of glycogen synthesis and degradation in muscle and other tissues. At present there appear to be two reasons for considering a cyclic mechanism involving the uridine-linked pathway for synthesis and phosphorylase for degradation: (1) the experiments on phosphorylase activation associated with glycogen breakdown and (2) the present experiments indicating that glycogen synthesis occurs at ratios of inorganic phosphate to glucose-1-phosphate highly unfavorable to synthesis via the phosphorylase system.

The physiological significance of this over-all system may be considered in terms of the two following mechanisms: (1) a system lending itself much more readily to control mechanisms and (2) a *p*H-dependent system in which glycogen synthesis would be highly favored thermodynamically at neutral *p*H. Another question raised by the present experiments is the following: Can the increased glycogen synthesis observed in these experiments in the presence of insulin be explained by increased penetration of glucose alone? Three findings argue against this thesis; (1) the high glycogen yields in terms of glucose uptake in the balance experiments; (2) the failure of net glycogen levels to be increased at a medium glucose concentration of 280 mg. per 100 ml. under conditions where glucose uptakes were elevated; and the fact that there is (3) no elevation of the glucose-1-phosphate fraction with an elevated hexose-6-phosphate in the presence of insulin.

TABLE 7
UDPG PYROPHOSPHORYLASE ACTIVITY OF RAT MUSCLE EXTRACTS

	Spectrophotometric activity* mg./100 gm./hr.
	8430
	7800
	8820
	10000
	8110
	10650
	8600
	8150
	7670
	8420
Mean	8665 (48.1 mmoles)

* Reaction mixture contained (final concentration): UDPG, 2×10^{-4} M; Mg^{++}, 2×10^{-3} M; TPN^{+}, 3×10^{-4} M; glucose 1,6-diphosphate, 10^{-6} M; glucose-6-phosphate dehydrogenase, 0.01 ml. (0.01 unit); glycylglycine buffer, 0.025 M, *p*H 7.4; enzyme, 0.01 ml. of 1:3 extract of muscle; volume, 1 ml.; temperature, 37°C.

† Triphosphopyridine nucleotide.

While none of these alone is conclusive, all three taken together constitute evidence for the fact that the glycogen synthesis observed in the presence of insulin does not appear to be explained by the increased penetration of glucose alone. In view of the experiments of Shaw and Stadie[19] and the demonstration of the activation of the UDPG glycogen transglucosylase by added glucose-6-phosphate, two factors may be considered. Further experiments will be required to assess the significance of these factors in the relationship of glycogen synthesis to the transport action of insulin in muscle.

Summary

Balance experiments carried out with rat diaphragms after short-term experiments at 2 concentrations of medium glucose indicate that more than 90 per cent of the increased glucose disappearing from the medium in the presence of insulin can be accounted for as tissue glycogen. The increase in glycogen

occurs in a perchloric acid-soluble fraction, with a smaller increase in the residual fraction at the higher medium glucose level.

Inorganic phosphate, phosphocreatine, ADP, and ATP are unchanged under these conditions, which is compatible with an insulin-stimulated turnover of high-energy phosphate compounds. Under our conditions hexose-6-phosphate content, but not glucose-1-phosphate content, is increased in insulin-stimulated diaphragms. Insulin-stimulated glycogen synthesis occurred at ratios of inorganic phosphate to glucose-1-phosphate of 305.

Enzyme activities measured in muscle extracts indicate the presence of UDPG pyrophosphorylase and UDPG transglucosylase in sufficient activity to account for the insulin-stimulated glycogen synthesis. The question of a cyclic mechanism of glycogen synthesis and degradation is discussed. Evidence is presented indicating that the insulin-stimulated glycogen synthesis does not appear to be explained solely by the increased penetration of glucose.

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REGULATION OF GLUCOSE UPTAKE BY MUSCLE: FUNCTIONAL SIGNIFICANCE OF PERMEABILITY AND PHOSPHORYLATING ACTIVITY*

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Much information concerning the pathways and individual enzymes involved in carbohydrate metabolism has been obtained from studies with cell-free systems. Similar studies with intact cells or multicellular systems, however, have frequently yielded results that appear to be at variance with this information. It is because of these discrepancies that there is an increasing awareness of the importance of structural elements for biochemical events in the intact cell. What, for example, are the functional limitations imposed by cell structure on enzymatic processes? Progress has been slow in this area primarily because of the lack of adequate techniques for measuring discrete reactions within intact cells. The ultimate goal of such an approach is, of course, an understanding of the processes that determine the integration of enzymatic activities at the cellular level of organization.

Since every intact cell possesses a confining membrane, one of the first questions we may ask is: How does the permeability of this structure influence cellular metabolism? Are the rates of intracellular penetration of cellular nutrients, such as sugars and amino acids, a determinant in their rate of utilization, or are the intracellular enzymatic processes, for which these compounds are substrates, the rate-limiting events? Recently, considerable attention has been focused on the process of sugar entry into muscle as a possible site of regulation of carbohydrate metabolism of muscle.[1] How sugars penetrate the muscle cell is not clearly understood, but available evidence suggests that at least two mechanisms are involved: one possessing the characteristics of simple diffusion[2] and the other described by the kinetics of an active transport process.[3, 4] Unfortunately, measurement of the penetration of glucose independent of subsequent enzymatic steps has not been feasible. For this reason, a variety of poorly metabolizable sugars have been used as glucose models to study the transfer of sugars across the muscle cell membrane and the effect thereon of various hormones, dietary regimens, and muscular activity.[5-9] The fact, however, that these sugars are poorly metabolized limits their usefulness in clarifying the interrelationship between the processes of penetration and utilization. Such information is required in order to support the concept that glucose entry is rate limiting and the site of insulin action.[1]

The present study is concerned with an analysis of the functional importance of permeability and phosphorylating activity as regulators of glucose utilization by muscle. This analysis was accomplished by the use of two different experimental procedures. The first method consisted of determining the distribution of free glucose between the extracellular and intracellular water of muscle. It was assumed that a glucose distribution greater than the extracellular space, as measured with raffinose, sucrose, or thiosulfate, denoted intracellular accumulation, whereas an equal or smaller value indicated that glucose was confined to the extracellular water. The intracellular concentration of glucose is an index of the relative rates of glucose penetration and phosphorylation by hexokinase. An increase in the intracellular concentration of the free sugar can result either from a relative increase in the rate of penetration or from a relative decrease in the rate of phosphorylation. In either circumstance, the presence of glucose within the cell would indicate that phosphorylation was rate limiting. On the other hand, the absence of free sugar from the intracellular compartment would mean that the transport of glucose across the cell membrane was the rate-limiting step in glucose utilization.

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* The work reported in this paper was supported in part by Grant A-1921 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† John and Mary R. Markle Scholar in Medical Science.

The second method consisted of measuring the rate of uptake of the glucose analogue 2-deoxyglucose by two different *in vitro* rat diaphragm preparations: (1) the usual isolated rat hemidiaphragm described by Gemmill[10] and referred to in this paper as the *cut* preparation, and (2) the *intact* diaphragm preparation developed in our laboratory.[2] A more detailed discussion of the physiological and metabolic properties of these preparations will be given in a subsequent section. The 2-deoxyglucose is phosphorylated by hexokinase, K_m — 2.4×10^{-5}M,[11] to form the phosphate ester 2-deoxyglucose-6-phosphate, which accumulates within the cell, since it is not acted upon at an appreciable rate by other muscle enzymes. Methods have been developed for the determination of free and phosphorylated 2-deoxyglucose in the presence of each other,[4] thereby permitting the investigation of intracellular penetration and phosphorylation as separate processes.

Effect of Insulin and Epinephrine on the Glucose Distribution in the Diaphragm and Gastrocnemius of Nonfasted Rats

Glucose was confined to the extracellular space in both the gastrocnemius and diaphragm of the nonfasted rats (TABLE 1, columns 1 and 7) at plasma glucose levels ranging from 98 to 163 mg. per cent. Intracellular accumulation did occur, however, following the injection of epinephrine (TABLE 1, columns 2 and 8). This was not a result of hyperglycemia per se, for glucose remained extracellular when correspondingly high plasma levels were attained by the intravenous administration of a glucose load (TABLE 1, column 3). Insulin, on the other hand, had a variable effect, depending on the degree of hypoglycemia induced. With moderate hypoglycemia, the percentage of distribution of glucose in the extracellular water was decreased (TABLE 1, column 4). With severe hypoglycemia (TABLE 1, column 5), which results in an endogenous discharge of epinephrine, glucose accumulated within the muscle cell. A concomitant effect of epinephrine secretion is the intracellular accumulation of glucose-6-phosphate,[12, 13] a noncompetitive inhibitor of hexokinase (K_i — 4×10^{-4} M)[14] arising from an accelerated rate of glycogenolysis. It seems probable that the accumulation of intracellular glucose occurring during epinephrine secretion resulted from an inhibition of phosphorylation secondary to the elevated phosphate ester concentrations ($> 2 \times 10^{-3}$M) observed under these conditions. The intracellular accumulation of glucose associated with severe insulin hypoglycemia could then be explained as reflecting an accelerated rate of glucose entry (insulin effect) and a decreased rate of phosphorylation (epinephrine effect). Consonant with this interpretation is the finding that insulin administered simultaneously with epinephrine did not counteract the effect of the latter on glucose distribution, but increased the intracellular accumulation of free sugar (TABLE 1, columns 6 and 10).

Variations in the endogenous secretion of epinephrine have been avoided by adrenalectomizing animals 30 to 60 min. before the experimental period; this procedure, in essence, depletes the animal of epinephrine without producing adrenocortical insufficiency. In these rats insulin had only one effect even when hypoglycemia was severe, namely, a marked depletion of glucose in the extracellular space of muscle (TABLE 1, column 9). This depletion suggests that, under conditions of rapid uptake and low plasma glucose levels, the diffusion of sugar in the interstitial fluid from the capillaries to the cell surface may become rate limiting. Of further interest is the finding that intracellular glucose did not accumulate in acutely adrenalectomized rats given insulin and large glucose loads sufficient to maintain hyperglycemic levels averaging 389 mg. per cent (TABLE 4). This would suggest that the phosphorylating capacity of muscle in the fed animal is very large and not readily exceeded by penetration, providing epinephrine secretion is prevented.

TABLE 1*

INFLUENCE OF EPINEPHRINE AND INSULIN ON GLUCOSE DISTRIBUTION IN THE DIAPHRAGM AND GASTROCNEMIUS OF THE NONFASTED RAT**

Column No.	Epinephrine†	Insulin††	Diaphragm			Gastrocnemius		
			C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (percentages)	C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (percentages)
Intact animal								
1	−	−	130 (6)	35.4	27.2	163 (10)	25.1	15.4
2	+	−	354 (3)	115.2	32.5	190 (4)	67.4	35.7
3‡	−	−	262 (3)	72.4	27.6	288 (5)	43.0	14.9
4	−	+	66 (5)	13.6	20.6	64 (5)	7.9	12.3
5	−	+	—	—	—	53 (5)	13.8	26.2
6	+	+	248 (3)	133.9	54.0	—	—	—
Acute adrenalectomy§								
7	−	−	98 (4)	20.4	20.8	112 (3)	13.2	11.8
8	+	−	372 (2)	129.5	34.8	155 (1)	51.0	32.8
9	−	+	61 (4)	6.2	10.2	40 (3)	3.1	7.9
10	+	+	264 (3)	131.5	49.8	—	—	—

* Reproduced from Kipnis *et al.*[13] by permission from *The Journal of Biological Chemistry.*

** The glucose distribution values ($C_m/C_p \times 100$) are to be compared with a raffinose space of 27.3 per cent for diaphragm and of 20.4 per cent for gastrocnemius. C_m and C_p refer to the glucose concentration in muscle and plasma water, respectively. The number of experiments is given in parentheses.

† Epinephrine (0.1 to 0.2 mg. per 100 gm. body weight) administered subcutaneously 30 min. before the removal of blood and muscle specimens.

†† Insulin (1.0 to 2.0 units per 100 gm. body weight) administered intraperitoneally 30 min. before the removal of blood and muscle specimens.

‡ Glucose loads of 150 to 250 mg./100 gm. body weight were administered intravenously. Twenty minutes later muscle and blood specimens were removed for analysis.

§ Bilateral adrenalectomy was performed 30 to 60 min. before the experimental period.

Effect of Insulin and Epinephrine on the Glucose Distribution in the Intact Diaphragm Preparation of Nonfasted Rats

Glucose could not be demonstrated inside the muscle cell of the *intact* diaphragm either in the presence or absence of added insulin (TABLE 2). Because of the remarkable similarity between the glucose distribution in the diaphragm of the acutely adrenalectomized rat and that in the *intact* preparation in the presence and absence of insulin (TABLE 2), it occurred to us that the *in vitro* diaphragm may represent an epinephrine-depleted muscle. If this were the case, the addition of epinephrine should result in the intracellular accumulation of glucose, an effect that would be reinforced in the presence of added insulin. This has been observed experimentally (TABLE 2).

Recent studies with the *intact* preparation have shown that the increase in glucose-6-phosphate concentration following epinephrine addition exactly paralleled the accumulation of intracellular glucose, and was of sufficient magnitude to account for at least a 70 per cent inhibition of hexokinase. Additional evidence indicating that epinephrine is capable of causing an inhibition of phosphorylation is presented in TABLE 3. In these experiments, the rates of 2-deoxyglucose penetration and phosphorylation were determined in the presence of insulin and epinephrine. Although phosphorylation was inhibited about 50 per cent, sugar entry was not affected.

TABLE 2

INFLUENCE OF EPINEPHRINE AND INSULIN ON GLUCOSE DISTRIBUTION IN THE *IN VITRO INTACT* DIAPHRAGM PREPARATION*

Temperature (centigrade)	Distribution of glucose in tissue water	
	C (percentages)	*I* (percentages)
37°	18.1 ± 0.9 (20.8)	11.1 ± 1.2 (10.2)
27°	21.7 ± 1.6	15.9 ± 0.8
27°†	37.2 ± 2.6 (34.8)	49.6 ± 3.5 (49.8)
17°	29.2 ± 1.3	23.3 ± 1.1

* Diaphragms were incubated for 30 min. in 0.01 M glucose at varying temperatures. *C* refers to incubation without and *I* to incubation with insulin (0.4 units per cc. of medium). The values represent the averages of 4 to 7 experiments. The numbers in parentheses are the values obtained *in vivo*.

† Epinephrine added (2.7×10^{-4} M) after 15-min. incubation in 0.02 M glucose. Incubation was then continued for an additional 5 min. before removal of the diaphragm for analysis.

Influence of Fasting and Glucose Loading on Glucose Distribution in Rat Diaphragm In Vivo

Glucose was confined to the extracellular space of the diaphragm in rats fasted up to 72 hours (TABLE 4). More prolonged periods of starvation, however, were associated with the appearance of free glucose in the muscle cell of both the diaphragm and gastrocnemius. In order to avoid variations in the rate of endogenous epinephrine secretion, which is known to be influenced by starvation,[13] the remainder of the experiments recorded in TABLE 4 were performed in acutely adrenalectomized rats.

Although insulin produced a depletion of extracellular glucose in the 24-hour-fasted adrenalectomized rat identical to that observed in the nonfasted control (TABLES 1 and 4), it was still possible to demonstrate that this short period of fasting produced a marked metabolic change in muscle. Plasma glucose levels averaging 389 mg. per cent were not associated with the intracellular accumula-

TABLE 3*

EFFECT OF EPINEPHRINE ON THE PENETRATION AND PHOSPHORYLATION OF 2-DEOXYGLUCOSE†

Period of incubation (min.)	2-Deoxyglucose 6-phosphate (μmoles/cc.)	2-Deoxyglucose (μmoles/cc.)	Total uptake (μmoles/cc.)
30	19.9	10.7	30.6 (33.6)
45	25.1	15.4	40.5 (41.5)

* Reproduced from Kipnis and Cori[2] by permission from *The Journal of Biological Chemistry.*

† Diaphragms were incubated in 0.06 M 2-deoxyglucose with insulin (0.4 units per cc.) added to increase the rate of penetration. The concentration of epinephrine was 6×10^{-5} M. The values are averages of 3 experiments. The values in parentheses represent the uptake of 2-deoxyglucose in the absence of epinephrine.

TABLE 4*

INFLUENCE OF FASTING AND OF GLUCOSE LOAD ON DISTRIBUTION OF GLUCOSE IN RAT DIAPHRAGM**

Length of fasting period (hours)	Insulin†	Glucose load‡	C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (percentages)
Intact animal					
24			122 (6)	32.8	26.9
72			108 (3)	25.5	26.4
120			114 (3)	34.1	29.8
Adrenalectomized animal§					
24			116 (3)	23.8	20.6
24	+		66 (3)	4.4	6.7
0		+	400 (4)	89.9	22.5
0	+	+	389 (4)	108.0	27.8
24		+	380 (3)	102.3	26.9
24	+	+	500 (3)	311.0	60.2
72		+	225 (3)	77.5	34.2
72	+	+	239 (3)	112.8	47.2
96		+	342 (3)	177.5	51.8
96	+	+	328 (3)	230.8	70.4

* Reproduced from Kipnis *et al.*[13] by permission from *The Journal of Biological Chemistry.*

** The glucose distribution values ($C_m/C_p \times 100$) are to be compared with a raffinose space of diaphragm of 27.3 per cent. C_m and C_p refer to the glucose concentration in muscle and plasma water, respectively. The number of experiments is given in parentheses.

† Insulin (1.0 to 2.0 units/100 gm. of body weight) was given by intraperitoneal injection 30 min. before the removal of blood and muscle specimens.

‡ Glucose loads varying from 150 to 250 mg./100 gm. of body weight were introduced by injection into the femoral vein; 20 min. later muscle and blood specimens were removed for analysis.

§ Bilateral adrenalectomy was performed 30 to 60 min. before the experimental period.

tion of glucose in nonfasted rats even after insulin administration. In 24-hour-fasted animals, however, similar conditions resulted in the accumulation of large quantities of glucose within the muscle cell. Furthermore, in animals fasted for from 72 to 96 hours, injection of glucose alone produced this effect, which was reinforced by the simultaneous administration of insulin.

It is known that prolonged starvation results in an impaired glucose tolerance.[15, 16] Obviously, this impairment is not a result of epinephrine secretion, nor is it secondary to an impairment of glucose entry, since intracellular glucose accumulated in the fasted animal. It would appear from these findings that the phosphorylating capacity of muscle is severely impaired by starvation. Preliminary studies suggest that this effect is mediated via pituitary and adrenal cortical secretions.

TABLE 5*

GLUCOSE DISTRIBUTION IN DIAPHRAGMS OF ALLOXAN-DIABETIC RATS**

Length of fasting period (hours)	Other experimental procedures	C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (percentages)
0	—	686 (4)	191	27.8
0	Insulin†	750 (3)	321	42.8
24	—	568 (5)	154	27.1
24	Insulin	340 (4)	158	46.5
24	Adrenalectomy‡	510 (3)	144	28.4
0	Adrenalectomy‡ + insulin†	315 (3)	138	43.8
0	Epinephrine§	650 (2)	303	46.6

* Reproduced from Kipnis *et al.*[13] by permission from *The Journal of Biological Chemistry.*

** The glucose distribution values ($C_m/C_p \times 100$) are to be compared with a raffinose space of 27.3 per cent. C_m and C_p refer to the glucose concentrations in muscle and plasma water, respectively. The number of experiments is given in parentheses.

† Insulin (1.0 to 2.0 units/100 gm. body weight) was administered intraperitoneally 30 min. before the removal of blood and muscle specimens.

‡ Bilateral adrenalectomy performed 30 to 60 min. before the experimental period.

§ Epinephrine (0.1 to 0.2 mg./100 gm. body weight) was administered subcutaneously 30 min. before the removal of blood and muscle specimens.

Influence of Alloxan Diabetes on Glucose Distribution in Diaphragm In Vivo

The effects of fasting have often been compared to the diabetic state and, as shown in TABLE 5, there is a considerable similarity in the results obtained with alloxan diabetic and fasted animals. In the untreated diabetic animal, in spite of very high plasma glucose levels, glucose distribution remained extracellular. The administration of insulin to the diabetic animal, whether fed, fasted, or acutely adrenalectomized, resulted in the marked accumulation of intracellular glucose. This response is in striking contrast to that observed in the nonfasted normal rat, in which no intracellular glucose could be measured even after the administration of insulin and a glucose load sufficiently large to sustain hyperglycemic levels comparable to those in the diabetic animal. It seems, therefore, that the phosphorylating capacity of diabetic muscle is diminished. Whether this is a primary effect of insulin deficiency or is secondary to other factors is discussed in a later section. The effect of epinephrine injection on intracellular distribution is the same in the diabetic as in the normal animal.

Uptake of 2-Deoxyglucose by the Cut- and Intact-Diaphragm Preparations

For purposes of presentation, the relative rates of 2-deoxyglucose uptake with and without insulin in the *cut* and *intact* preparations from normal (nonfasted), diabetic, diabetic-adrenalectomized, and hypophysectomized (fasted) rats are recorded in FIGURE 1. The uptake of 2-deoxyglucose by each group has been compared on a percentage basis with the diaphragm of the normal nonfasted animal without added insulin. The results with the *cut* preparation are similar to those reported for glucose uptake by numerous investigators.[17-20] The 2-deoxyglucose utilization of the *cut* diaphragm of the alloxan diabetic rat is reduced to about one half that obtained with normal muscle. Although insulin stimulated utilization in this preparation, the final rate attained is significantly lower than that of normal insulinized muscle. The *cut* diaphragm of the diabetic-adrenalectomized animal (adrenalectomy was performed 4 to 7 days after the diabetes was established and 4 days before the animal was sacrificed) was indistinguishable from that of the normal rat either in the presence or absence of insulin. Hypophysectomy resulted in a significant increase in

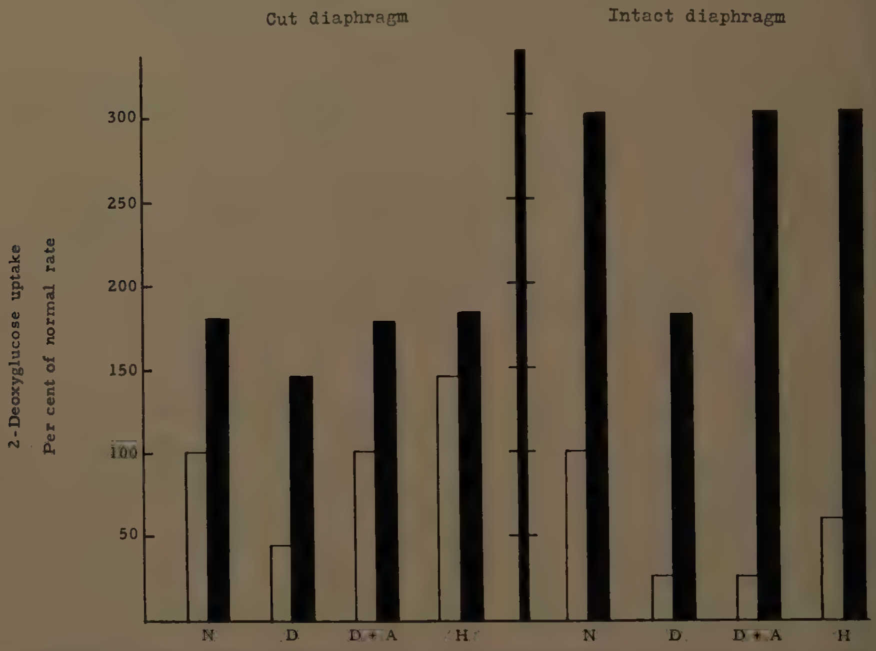

FIGURE 1. The effect of various hormone deficiencies on 2-deoxyglucose uptake by the isolated *cut* and *intact* diaphragm preparations. Each group has been compared with the normal diaphragm without added insulin. Uptake without insulin is shown by open bars; with insulin, by solid bars. N = normal (nonfasted); D, alloxan diabetic; D + A, alloxan diabetic-adrenalectomized; H, hypophysectomized (fasted).

the basal rate of utilization and a proportionately decreased stimulation by insulin.

The results obtained with the *intact* diaphragm differ markedly from those of the *cut* preparation. The stimulatory effect of insulin on 2-deoxyglucose uptake is greater in the *intact* than in the *cut* preparation. Furthermore, the *intact* diaphragm of the alloxan-diabetic rat exhibited a far greater impairment of uptake than is observed in the *cut* preparation. Insulin failed, as it did with the *cut* preparation, to restore the rate of uptake of the diabetic *intact* diaphragm to the level observed in normal insulinized muscle. The most striking differences were seen with the diabetic-adrenalectomized and hypophysectomized (fasted) animals. Contrary to the findings with the *cut* preparation, adrenalectomy, in the absence of insulin, did not alter the severely impaired utilization characteristic of the *intact* diaphragm of diabetic animals. In the presence of insulin, however, the rates of utilization were similar to those of normal insulinized muscle. Hypophysectomy was associated, in the *intact* preparation, with a significant decrease in the basal uptake of 2-deoxyglucose and a dramatic response to the addition of insulin.

Before discussing the last group of experiments, it is first necessary to examine the physiological properties of the *cut* and *intact* preparations and consider their metabolic implications. The usual isolated rat hemidiaphragm[10] has been referred to in this paper as the *cut* preparation because all of its muscle fibers are *cut* at both ends during its excision from its attachments to the rib cage, spine, sternum, and central tendon. The *cut* diaphragm has been used to study a variety of metabolic problems of muscle that involve the penetration as well as the utilization of sugars. A series of studies designed to determine whether the *cut* diaphragm is a suitable *in vitro* test object for permeability measurements led to the conclusion that it is inadequate for this purpose.[2, 21] For example, a variety of substances that are normally excluded from the cell interior, such as sucrose, raffinose, thiosulfate (FIGURE 2), inulin, and ferricyanide, readily penetrate into the intracellular compartment of the *cut* diaphragm. These shortcomings are not associated with the *intact* diaphragm, since none of the muscle fibers are damaged in its preparation. This preparation is similar to the diaphragm in the living animal with respect to total tissue water, intracellular and extracellular spaces (FIGURE 2), insulin responsiveness, and rates of sugar penetration.[2]

We have recently studied, in collaboration with Dr. Adolph Cohen, the electron microscopic appearance of the *intact* and *cut* preparations following prolonged incubation in Krebs-Henseleit phosphate buffer. The *intact* preparation could not be distinguished from the diaphragm removed from the living animal and immediately fixed for study; the *cut* preparation, on the other hand, exhibited severe and progressive cellular deterioration. The mitochondria were swollen and contained fragmented cristae, the muscle fibrils were thickened and indistinct, and the endoplasmic reticulum was markedly engorged.

The rates of intracellular penetration of both metabolizable and nonmetabolizable sugars are much faster in the *cut* preparation than in the *intact* one. For example, the cellular entry of 2-deoxyglucose is fourfold and that of D-xylose is tenfold more rapid in the *cut* than in the *intact* diaphragm. Not only are the permeability characteristics to sugars affected, but Menozzi *et al.*[22] have recently shown that ion transport is also markedly altered. Serious doubt, therefore, may be entertained as to the functional significance of the cell membrane as a metabolic regulator in the *cut* preparation. For example, no difference in permeability could be observed between the normal and the diabetic *cut* preparations using the nonmetabolizable insulin-responsive pentose D-xylose, even though the utilization of glucose and 2-deoxyglucose was decreased 50 per cent (FIGURE 3). In view of these findings, it appears likely that the utilization rates observed with the *cut* preparation are representative of the phosphorylating capacity of muscle, whereas those obtained with the *intact* preparation are a measure of the rate of intracellular transport.

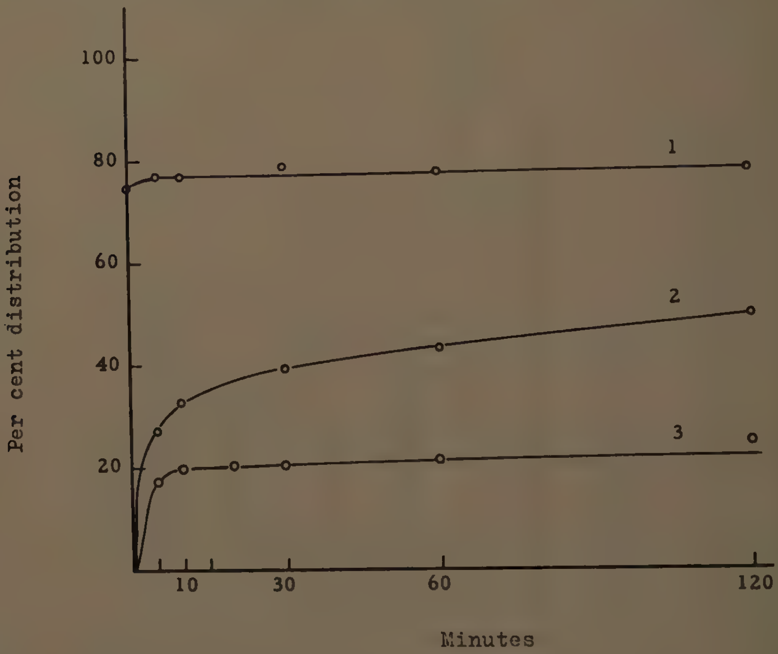

FIGURE 2. Total tissue water and extracellular space of the *cut* and *intact* diaphragm preparations. Total water content of both preparations was measured by drying to constant weight (curve 1). Extracellular space was measured with raffinose and thiosulfate in the *cut* diaphragm (curve 2), and with sucrose and thiosulfate in the *intact* diaphragm (curve 3).

The results that have been presented indicate that other factors, in addition to the permeability properties of the cell membrane, may limit the rate of glucose metabolism in muscle. Since the first intracellular event in glucose metabolism is its phosphorylation by hexokinase, it is reasonable to expect that this reaction may function as one of the regulators of glucose utilization. The fact that free glucose can be demonstrated within the cell in a variety of conditions supports this contention. For example, epinephrine, whether introduced by injection or discharged from the adrenals in response to hypoglycemia or other stimuli, such as muscle work, leads to an intracellular accumulation of free sugar. As mentioned previously, this reaction probably results from a decrease in the rate of phosphorylation caused by the associated elevation in the glucose-6-phosphate concentration. The decreased glucose tolerance associated with fasting is, in part, a reflection of a diminished rate of phosphorylation in muscle, since free sugar accumulates intracellularly under conditions in which it would have remained extracellular in the nonfasted animal. The muscle of the diabetic animal, in addition to a defective sugar transport system, is also characterized by an impaired phosphorylating capacity. Thus, free sugar accumulates within the cell, both *in vivo* and *in vitro*, upon the addition of insulin. It would appear, however, that membrane permeability is more seriously affected by insulin deficiency than is the phosphorylating capacity, since the rate of 2-deoxyglucose utilization by the diabetic *intact* diaphragm preparation is 10 to 20 per cent of normal as compared to a 50 per cent decrease in the diabetic *cut* diaphragm. This is in agreement with the results of recent experiments by Field and Cori[23] in which the rates of glucose utilization in normal and diabetic animals were estimated by analyzing the entire carcass for free sugar after the injection of a C^{14}-labeled glucose load. The diabetic animal metabolized glucose at 10 to 15 per cent of the rate observed in the normal control. The failure of insulin to correct immediately the depressed level of phosphorylation, either *in vivo* or *in vitro*, suggests that this defect is a secondary effect of insulin deficiency.

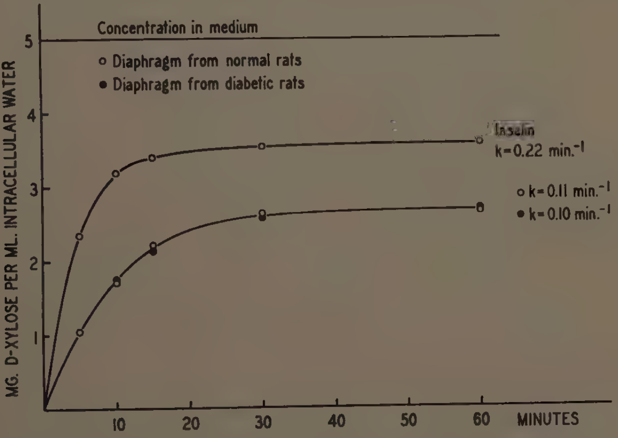

FIGURE 3. The rate of D-xylose penetration in the *cut* diaphragm of normal and alloxan diabetic rats.

It is possible to reconcile the divergent results obtained with the *cut* and *intact* preparations of the diabetic-adrenalectomized and hypophysectomized rats by supposing that the pituitary and the adrenal cortical secretions depress the phosphorylating capacity of muscle, but do not operate on the initial step in glucose uptake, namely, its entry into the cell. In support of this thesis are our findings (unpublished observations) and those of Levine[1] that adrenal steroids do not affect the intracellular penetration of metabolizable sugars such as 2-deoxyglucose and nonmetabolizable sugars such as D-xylose. One would not expect to observe any effect of the injection or removal of adrenal or pituitary secretions on glucose uptake under conditions where penetration was rate limiting. This observation is consistent with the results of Long and Lukens[24] and of others[25-27] who have shown that the carbohydrate tolerance of depancreatized animals is not improved by the removal of the adrenals. It is also in agreement with clinical observations that diabetes can occur in individuals with adrenal cortical insufficiency, and that the impaired glucose tolerance of the diabetic human is not corrected by adrenalectomy. Under conditions where penetration is not rate limiting, however, the effects of adrenal cortical ablation become evident. This is the situation in the diabetic *cut* diaphragm either with or without added insulin and in the diabetic *intact* preparation with added insulin. These results indicate that the impaired phosphorylating capacity of diabetic muscle is, at least in part, a reflection of adrenal cortical activity.

Houssay and his associates, in their classic studies on the effects of hypophysectomy on carbohydrate metabolism,[28-30] demonstrated that the hyperglycemic response to a glucose load was prolonged, that the extrahepatic consumption of sugar was decreased, and that the sensitivity to insulin was markedly increased. These original observations have since been confirmed in the living animal by numerous investigators using a variety of experimental techniques.[31-35] Similar results have now been obtained with the *intact* diaphragm. Since penetration is rate limiting in this *in vitro* preparation, the impaired uptake of sugar can be attributed to a diminished rate of transfer across the cell membrane reflecting, I believe, the decreased levels of insulin activity in the hypophysectomized animal.[36] These findings, however, are in contrast to the increased rate of utilization and the decreased insulin response observed in the *cut* diaphragm of the hypophysectomized animal. These results would be anticipated if, as previously proposed, the pituitary secretions depress phosphorylating activity, since utilization rates in the *cut* diaphragm reflect its phosphorylating capacity.

The intracellular accumulation of free sugar when transport is markedly accelerated by insulin or muscular activity suggests that the phosphorylating capacity of muscle functions as a protective restraint against excessive glucose utilization. The removal of this restraining influence would then be one of the factors contributing to the increased insulin sensitivity of the adrenalectomized or hypophysectomized animal.

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ROLE OF INSULIN IN TWO PATHWAYS OF GLUCOSE METABOLISM: *IN VIVO* GLUCOSAMINE AND GLYCOGEN SYNTHESIS*

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One manner of further localizing the action of insulin is to compare its role in the regulation of two pathways of glucose metabolism.

For this purpose, a comparison was made of the biosynthesis of liver glycogen and protein-bound glucosamine in the insulin-deficient state. This was done by measuring the incorporation of radioactivity from C^{14}-labeled glucose into the liver glucosamine and glycogen of the intact alloxan diabetic rat and comparing these values to those previously reported for the normal rat.[1]

It was of interest to compare the synthesis of liver glycogen to that of glucosamine in particular because an enzyme has been described in liver that synthesizes glucosamine from glucose-6-phosphate,[2] and also because it has been shown in a previous study that the liver is the principal site of synthesis of the serum glucosamine.[1] Glucosamine is present in the serum in large amounts as a major carbohydrate component of the serum glycoproteins, which have been shown to undergo wide fluctuation in concentration in a variety of pathological states.[3]

A study of the biosynthesis of glucosamine from C^{14}-labeled glucose in the normal intact rat indicated a very rapid turnover of this substance, with a turnover time of 0.8 hour for the liver glucosamine and 2.0 hours for the serum glucosamine. The following scheme was proposed to show the relationships between the serum glucose and the liver and serum glucosamine:[1]

$$\begin{tikzcd}
 & {} & & \\
\text{Serum glucose} \arrow[r, "\overset{v_1}{0.9\ \mu\text{M/hr.}}"] & \text{liver glucosamine} \arrow[u] \arrow[r, shift left, "\overset{v_2}{25.6\ \mu\text{M/hr.}}"] & \text{serum glucosamine} \arrow[l, shift left, "26.3\ \mu\text{M/hr.}"] \arrow[r, "v_5\ ?"] & {}
\end{tikzcd}$$

From the rates indicated for a 250-gm. rat it may be seen that, in addition to the synthesis of new glucosamine molecules from glucose, a very rapid exchange takes place between the glucosamine of liver and serum.

The data given in the present study show that in diabetic rats the biosynthesis of glucosamine from glucose is essentially unimpaired, despite an almost complete inability of these animals to synthesize liver glycogen from glucose.

Experimental

Male albino rats of the Wistar strain weighing between 220 and 320 gm. were used. All animals were fed Purina Chow ad libitum prior to as well as during the experiment to the time of sacrifice. Diabetes was produced by the intravenous injection of alloxan monohydrate (37 to 39 mg./kg.) after a 24-

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* The work reported in this paper was supported in part by a Postdoctoral Fellowship from the American Cancer Society, Inc., New York, N. Y.

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hour fast. The animals were used for the experiments between 1 and 4 months after alloxan injection, and then only if their blood glucose was greater than 300 mg. per cent. Each animal was given a single intraperitoneal injection of 10 to 15 μc. glucose-U-C^{14} (2.08 μc./mg.) in 2 ml. of physiological saline. At various time intervals following the injection of the radioactive glucose, 0.2 ml. of blood was taken from the tail vein of each rat without anesthesia for the purpose of determining the blood glucose specific activity. The animals were sacrificed at various times following the injection of the glucose-C^{14} by exsanguination by way of the inferior vena cava under light ether anesthesia.

The details of the isolation procedures, as well as of the chemical and isotopic analyses, were identical to those previously described for normal animals.[1] The protein-bound glucosamine was isolated from the trichloracetic acid precipitates of liver, kidneys, lungs, testes, and spleen, and from the ethanol precipitate of serum. The glucosamine was liberated from the protein precipitates by acid hydrolysis and then separated from neutral sugars on a Dowex-50 cation exchange column. The amino sugar was then converted to the glucose phenylosazone for the purpose of determining its specific activity. The liver and kidney glycogen was precipitated from the trichloracetic acid extracts by the addition of ethanol and, following acid hydrolysis, the glucose phenylosazone was prepared for counting its radioactivity. Likewise, the activity of blood and urine glucose was determined from the glucose phenylosazone formed from the Somogyi filtrates.

Colorimetric and chromatographic analyses of the Dowex-50 eluates of hydrolyzed diabetic tissues revealed the same very high ratio of glucosamine to galactosamine that is found in normal tissues.[1]

All measurements of radioactivity were adjusted to 5.0×10^6 cpm injected into a 250-gm. rat.

Results

Incorporation of radioactivity into glucosamine. The specific activity and total activity of the protein-bound glucosamine of liver and serum following the injection of a tracer dose of glucose-C^{14} are shown in TABLE 1. From the ratio of the specific activity of the liver glucosamine to that of the serum glucosamine it may be seen that, while the liver is more active than the serum early in the course of the experiment, the serum activity soon becomes equal to and then exceeds that of the liver (TABLE 1). The activity in the protein-bound glucosamine of several other tissues was also determined at 3.75 hours following the injection of the glucose-C^{14} (TABLE 2).

Incorporation of radioactivity into liver and kidney glycogen. Very little activity was incorporated into the liver glycogen from glucose in any of the animals studied (TABLE 1). Very high levels of kidney glycogen were present (TABLE 2), in contrast to normal animals, which were found to have only about 2 μmole (glucose equivalents) of kidney glycogen. In the diabetic animals the specific activity of the kidney glycogen, as well as its total activity, was actually several times higher than that of the liver glycogen.

Comparison of normal and diabetic rats. In order to compare properly the incorporation of radioactivity into the glucosamine of normal and diabetic rats, it was necessary to take into account any differences in the specific activity of the blood glucose in these two types of animals. For this purpose, several samples of blood were obtained from each rat following the injection of glucose-C^{14}; the specific activities at each time are plotted in FIGURE 1.

TABLE 1

RADIOACTIVITY IN LIVER GLUCOSAMINE, SERUM GLUCOSAMINE, AND LIVER GLYCOGEN FOLLOWING GLUCOSE-C^{14} INJECTION INTO DIABETIC RATS

Rat No.	Time Hours	Liver glucosamine			Serum glucosamine		Liver-specific activity / Serum-specific activity	Liver glycogen	
		Specific activity cpm/μmole	Quantity* μmoles	Total activity cpm	Specific activity cpm/μmole	Total† activity cpm		Quantity* μmoles glucose equivalents	Total activity cpm
22	1.50	8.6	28.5	246	5.0	229	1.74	251	70.6
6	3.75	17.6	27.9	491	17.9	810	0.98	354	66.2
7	3.75	14.7	26.5	388	14.5	667	1.01	264	50.4
20	3.75	11.1	26.7	297	11.6	534	0.96	73	15.6
26	12.00	7.2	25.0	179	9.6	444	0.74	88	16.7
8‡	3.75	50.8	25.6	1312	49.3	2270	1.03	500	505.0

All counts are adjusted to 5.0 × 10^6 cpm injected into a 250-gm. rat.

* Quantity refers to the total μmoles in the liver of a 250-gm. rat.

† Mean serum glucosamine level: 4.6 ± 0.5 μmoles per ml. Total activity calculated from a mean pool of 46 μmoles per 250-gm. rat, assuming serum volume to be 4 per cent of body weight.

‡ Fructose-C^{14} injected instead of glucose-C^{14} under the same conditions.

TABLE 2

RADIOACTIVITY IN GLUCOSAMINE OF SEVERAL TISSUES AND IN KIDNEY GLYCOGEN FOLLOWING GLUCOSE-C^{14} INJECTION INTO DIABETIC RATS

Rat No.	Kidney				Lung		Testes		Spleen	
	Glycogen		Glucosamine							
	Quantity μmoles glucose equivalents	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm
6	66.6	83	8.34	117	30.1	293	5.13	19.1	14.1	18.7
7	75.5	116	6.26	86	11.0	83	6.35	21.7	8.1	15.5
20	197.0	156	5.53	75						

Animals sacrificed 3.75 hours following glucose-C^{14} injection. All values are adjusted to 5.0 × 10^6 cpm injected into a 250-gm. rat.

Total activity refers to the counts per minute in the glucosamine or glycogen of the tissues of a 250-gm. rat. The mean protein-bound glucosamine content of tissues in μmoles per 250-gm. rat was: kidney, 13.8; lung, 8.62; testes, 3.57; spleen, 1.62.

The diabetic animals had an average blood glucose level of 561 mg. per cent compared with 116 mg. per cent for the normal animals, and excreted an average of 142 mg. of glucose in the urine per hour per 100 gm. of body weight, causing a loss of 58 per cent of the injected radioactivity during the course of the experiment. The body glucose pools, calculated by the method of

Feller *et al.*,[4] were 207 mg./100 gm. of body weight for the diabetic animals and 53 mg./100 gm. body weight for the normal.

FIGURE 1 shows that the specific activity of the serum glucose of the normal animals is considerably greater than that of the diabetic animals. In order to compare the activity in the glucosamine and glycogen in the diabetic animals with the activity of these substances in the normal animals, it is first necessary to correct for the lower serum glucose-specific activity to which the tissues of the diabetic animals were exposed, due to both a greater dilution of the injected glucose-C^{14} and a slower decay of its specific activity. From the ratio of the area under the normal specific-activity curve to that under the diabetic specific-activity curve, the following correction factors were calculated: 3.83 for 1.5 hours, 3.18 for 3.75 hours, and 3.11 for 12 hours following the glucose injection.*

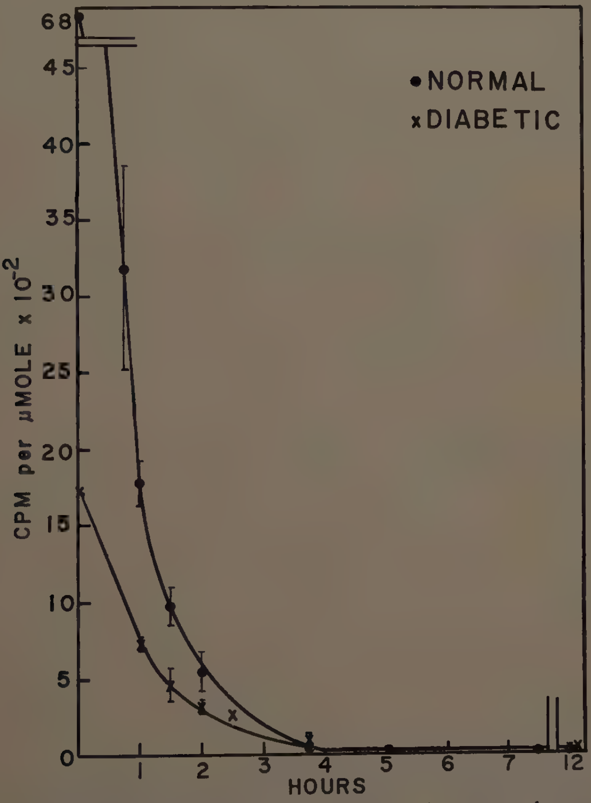

FIGURE 1. A comparison of the specific activity of the serum glucose of normal and diabetic animals at various times following the injection of glucose-C^{14}. The values expressed as counts per minute $\times 10^{-2}$ per μmole of glucose are plotted as the mean ± the standard error, and the value at 0 time was obtained from the intercept of the semilogarithmic plot of the specific activities for the first 2 hours.

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* Since the equations for the specific activity of a product at any time involve the integral of the specific activity of the precursor up to that time, the necessary correction factors for

As may be seen from TABLE 3, the pool sizes for the liver and serum glucosamine were essentially the same in normal and diabetic animals, whereas the liver glycogen pool was markedly reduced in the diabetic.

In TABLES 4 and 5 the diabetic values for both glucosamine and glycogen corrected to the normal serum glucose-specific activity by multiplication by the above factors are given alongside mean normal values for the same time intervals. At the time intervals shown, there is little difference between the total activity of the glucosamine of normal or diabetic animals either in liver or in serum. In marked contrast to this essentially normal synthesis of glucosamine from glucose by the diabetic liver, there was a pronounced impairment in the synthesis of liver glycogen from glucose (FIGURE 2). Glycogen activity in the diabetic liver was reduced to about 1 per cent of normal (TABLE 4). It may be noted that, while the normal animal incorporates manyfold more activity into the total liver glycogen than into the total liver glucosamine, the inverse is actually true in the diabetic animal (TABLE 4).

TABLE 3

COMPARISON OF POOL SIZES IN NORMAL AND DIABETIC RATS

Type of animal	Liver		Serum glucosamine
	Glycogen glucose equivalents	Glucosamine	
Normal ± s.e.	1,720 ± 191	21.5 ± 0.4	50.0 ± 2.0
Diabetic ± s.e.	206 ± 54	26.9 ± 0.6	46.0 ± 5.2

All values expressed as μmoles per 250-gm. rat.

TABLE 4

A COMPARISON OF RADIOACTIVITY IN LIVER GLYCOGEN, LIVER GLUCOSAMINE, AND SERUM GLUCOSAMINE OF NORMAL AND DIABETIC RATS

Time hours	Liver								Total serum glucosamine activity		
	Total glycogen activity			Total glucosamine activity			Glycogen/Glucosamine				
	Normal cpm	Diabetic cpm	Normal/Diabetic	Normal cpm	Diabetic cpm	Normal/Diabetic	Normal	Diabetic	Normal cpm	Diabetic	Normal/Diabetic
1.5	21,138	271	77.9	1157	941	1.23	18.3	0.29	1101	830	1.33
3.75	33,973	140	243.0	1021	1248	0.82	33.2	0.11	2500	2130	1.17
12.0	3,628	52	62.9	531	557	0.96	6.2	0.09	1527	1370	1.11

All counts adjusted to 5.0 × 10^6 cpm glucose-C^{14} injected into a 250-gm. rat.

All values are means for each time, and are expressed per 250-gm. rat; the normal values were reported previously.[1] All activities given for diabetic animals are corrected to the normal serum glucose-specific activity as explained in the text.

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liver glucosamine and glycogen activity were obtained from the geometrical integration of the specific activity of the blood glucose, which is represented by the area under the blood glucose-specific activity curve up to a given time.

As in liver and serum, the other diabetic tissues studied do not appear to show any impairment in the synthesis of glucosamine from glucose (TABLE 5) at the time studied.

TABLE 5

A COMPARISON OF THE RADIOACTIVITY IN THE GLUCOSAMINE OF SEVERAL TISSUES OF NORMAL AND DIABETIC RATS

Tissue	Total Activity		
	Normal cpm	Diabetic* cpm	Normal/Diabetic
Kidney	184†	294	0.63
Lung	250	585	0.43
Testes	70.3	54.4	1.29
Spleen	73.6	64.8	1.13

Animals sacrificed 3.75 hours following glucose-C^{14} injection. All counts adjusted to 5.0×10^6 cpm injected into a 250-gm. rat.

* All activities for diabetic animals are corrected to the normal serum glucose-specific activity, as explained in text.

† All values are means and are expressed per 250 gm. rat; the normal values were reported previously.[1]

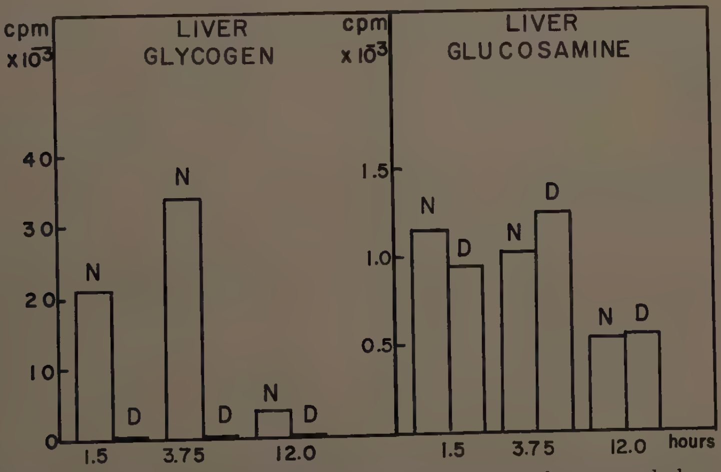

FIGURE 2. A comparison of the total activity in the liver glycogen and glucosamine of normal and diabetic rats. The values are means for each time and are expressed per 250-gm. rat. The activities of the diabetic animals are corrected to the normal serum glucose-specific activity.

Incorporation of radioactivity into glucosamine following injection of fructose-U-C^{14}. Fructose-U-C^{14} was injected into a diabetic rat under the same conditions as for glucose-U-C^{14}, and the animal was sacrificed 3.75 hours thereafter. As observed in normal rats,[1] both liver and serum glucosamine activity increased in the same proportion, so that the ratio of the two remained the same as with glucose (TABLE 1). No significance can be attached to the absolute increase, since the specific activity of the injected fructose would be diluted less than that of the injected glucose.

Discussion

It appears from the data of the present study that the synthesis of protein-bound glucosamine from glucose in several tissues of the alloxan diabetic rat is not significantly altered from the normal. Moreover, the relation of the specific activities of the liver and serum glucosamine to each other following the injection of C^{14}-labeled glucose or fructose (TABLE 1) seems to be essentially the same as that reported for the normal.[1] It is therefore quite likely that in the diabetic animal the liver is the primary site of synthesis of the serum glucosamine, as appears to be the case in the normal animal.[1]

It is of interest that Schiller and Dorfman have observed that the incorporation of radioactivity from C^{14}-labeled glucose into the hyaluronic acid of the skin of alloxan diabetic rats was reduced to about one third of normal.[5] This decreased activity of the hyaluronic acid molecule could represent a decreased synthesis of either the glucosamine or the glucuronic acid moieties, or both. Since these investigators did not measure the specific activity of the blood glucose, it is difficult to compare their results with the data obtained in the present study, in which the markedly decreased blood glucose-specific activity of the diabetic animals was taken into account.

The marked impairment in liver glycogen synthesis from glucose observed in the present study using the intact alloxan diabetic rat confirms the results obtained previously using liver slices from alloxan diabetic rats.[6, 7]

The observation that the liver of the intact alloxan diabetic rat can synthesize glucosamine from glucose at a normal rate despite an almost total impairment in the synthesis of glycogen suggests that insulin does not play the same role in the regulation of all of the products of glucose metabolism. That the impairment in glycogen synthesis in the alloxan diabetic rat is due to insulin deficiency has been demonstrated in a previous study.[7] In order to attempt to explain this difference in the effect of insulin deficiency on the synthesis of glycogen and glucosamine, it is necessary to consider the pathway of glucosamine synthesis from glucose. Pogell and Gryder have demonstrated the presence in rat liver of an enzyme that can synthesize glucosamine-6-phosphate from glucose-6-phosphate and glutamine.[2] If glucose-6-phosphate is also the precursor of glucosamine in the liver of the intact animal, it would appear likely that it belongs to a glucose-6-phosphate pool that is not under the influence of insulin and is therefore a pool separate from that giving rise to glycogen. If the action of insulin on the liver is one affecting the penetration of glucose, it may be that glucosamine synthesis takes place in a compartment of the liver cell separate from that in which glycogen synthesis takes place. However, if the action of insulin is on the phosphorylation of glucose, there may be two separate phosphorylating enzymes, only one of which is responsive to insulin. In this regard, it is of interest that Shaw and Stadie have demonstrated that, in the rat diaphragm, glycogen synthesis from glucose is responsive to insulin, whereas lactic acid formation from glucose is unaffected by insulin.[8] In order to explain their data, these investigators have postulated the existence of two separate glucose-6-phosphate pools, one inside the cell that is responsive to insulin, and another on the cell surface.

This difference in response to insulin deficiency of the liver glycogen and glucosamine is consistent with the observations made in normal animals, in which there was no correlation between the activity incorporated into the liver glycogen and glucosamine.[1]

However, it is also necessary to consider the possibility that there is only one glucose-6-phosphate pool, but that the enzyme responsible for the amination of glucose-6-phosphate has such a high affinity for its substrate that it remains saturated despite a decreased formation of glucose-6-phosphate from glucose, resulting in unimpaired glucosamine synthesis.

It is of interest to note that, like the liver, the other tissues reported in this study also synthesize glucosamine from glucose at essentially a normal rate. The data of this study may be of some relevance in evaluating the origin of the retinal and glomerular lesions found in human diabetes, which have been postulated to be the result of some disorder of mucopolysaccharide metabolism.[9]

Summary

A comparison was made of the biosynthesis from glucose of liver glycogen and protein-bound glucosamine in the intact alloxan diabetic rat. From the incorporation of radioactivity from tracer doses of glucose-U-C^{14} into these two substances, it was judged that there was no impairment in the rate of synthesis of the glucosamine despite an almost complete failure in the synthesis of glycogen. This difference in response to insulin deficiency of two pathways of glucose metabolism is discussed, and its possible relevance to localizing the site of action of insulin is considered.

The synthesis of the protein-bound glucosamine of serum, kidney, lung, testes, and spleen in the diabetic rat was also studied with the aid of glucose-C^{14} and, as in liver, no decrease from the normal was observed.

From the relationship of the specific activity of the liver and serum glucosamine at several time intervals, the liver appeared to be the primary site of synthesis of serum glucosamine, as has been reported for the normal rat.

Acknowledgment

I express my appreciation to A. Baird Hastings for his interest in this work.

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Part III. Biochemistry and Physiology of Insulin and the Sulfonylurea Compounds

EFFECT OF 6-DEOXY-6-FLUOROGLUCOSE ON GLUCOSE PERMEATION OF THE CELL*

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The processes involved in the permeation of glucose into muscle cells have received considerable attention in recent years. The possible ways of transfer may be roughly classified in two ways: (1) passage or diffusion in a chemically unmodified form, and (2) passage by the participation of a membrane carrier system possibly associated with coupling and uncoupling enzymes. It is questionable whether there is sufficient evidence at present to support one hypothesis to the exclusion of the other. The use of glucose analogues has shown that the uptake of hexoses by muscle cells can be limited by factors of steric specificity.[1] The use of modified sugars is not always a rewarding one, because one cannot always predict in advance whether or not a particular compound will be useful. Most of the compounds examined thus far have been of no value because of their inertness for one reason or another. However, 6-deoxy-6-fluoroglucose (hereafter referred to as 6-FG) is a compound with interesting physiological properties. In this report we present a summary of the effect of 6-FG on the intracellular transfer of glucose.

Preparation of 6-FG and Facts Regarding It

We prepared 6-FG according to the procedure of Helferich *et al.*[2, 3] as revised by Blakley.[4] This compound is a well-defined crystalline compound with the stereochemical configuration of glucose. In considering the possibility that this compound may enter into an "enzyme socket," it should be pointed out that the atomic radius of covalent fluorine is smaller than that of the hydroxyl group that it replaces.

Glucose Permeation and Inhibitory Action of 6-FG

The physiological properties of 6-FG as previously reported by us are summarized as follows:

(1) 6-FG rapidly enters the cells of the extrahepatic tissues and simultaneously inhibits the intracellular transfer of glucose.[5]

(2) The rate of cell entry of 6-FG and its final distribution in the tissue water of the extrahepatic tissues is not altered by the administration of insulin; likewise, the uptake of 6-FG by rat diaphragm *in vitro* is not increased by the presence of insulin in the incubation medium.[5]

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* The work reported in this paper was supported in part by Grant A-2949 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

(3) 6-FG inhibits the oxidation of uniformly labeled glucose-C^{14} in *in vitro* studies using rat kidney slices,[6] rat epididymal adipose tissue, and rat diaphragm tissue.[7]

A comparison of the relative effectiveness of 6-FG as an inhibitor of glucose oxidation in rat kidney and adipose and diaphragm tissues is shown in FIGURE

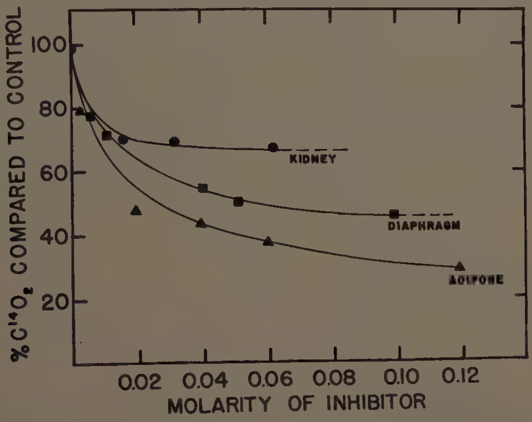

FIGURE 1. Comparison of the effect of 6-FG on the inhibition of glucose (uniformly labeled) oxidation on rat kidney, rat diaphragm, and rat epididymal adipose tissue.

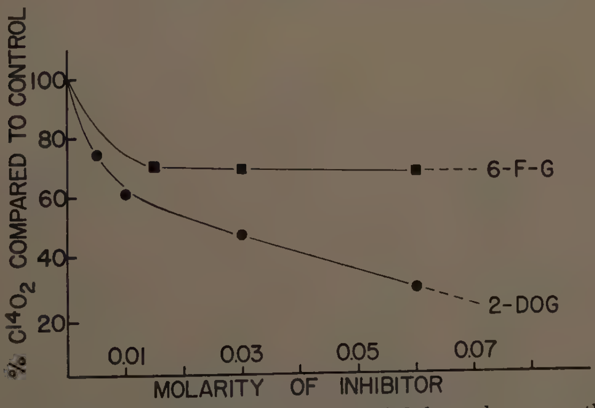

FIGURE 2. Comparison of the effect of 6-FG and 2-deoxyglucose on the inhibition of glucose (uniformly labeled) oxidation in rat kidney slices.

1. It is apparent from the data that, as an inhibitor of glucose oxidation, 6-FG is most active in adipose tissue in the series: adipose tissue > diaphragm > kidney.

The most complete data regarding the action of 6-FG on the entry of glucose into the cells are furnished by the results with kidney tissue. FIGURE 2 shows data on the effect of 6-FG concentrations on the conversion of uniformly labeled glucose to CO_2 with this tissue. With increasing concentration of 6-FG, the inhibition of radioactive CO_2 formation approaches a plateau.

On the other hand, 2-deoxyglucose maintains an increase in inhibition with increasing concentrations of the inhibitor. With adipose and diaphragm tissue, the 6-FG inhibition curves also approach a plateau, whereas the curves obtained with 2-deoxyglucose approach 100 per cent inhibition.[7] With respect to kidney tissue, the inhibitory action of 6-FG is qualitatively the same with C_1, C_6, or uniformly labeled glucose (FIGURE 3).

Modes of Cell Permeation as Suggested by These Studies on 6-FG

The plateau type of inhibition curve resulting from these studies is unusual. A possible explanation of these data is that 6-FG inhibits a specific pathway of glucose oxidation, but that one or more alternate pathways exist. Under these conditions increasing the concentration of 6-FG ultimately would result in complete inhibition of the susceptible pathway, while the alternate pathway would be unaffected. From this line of reasoning 2-deoxyglucose would have to inhibit at a point where all glucose oxidation to CO_2 is affected, which is in agreement with the information available for it.[8] If the point of glucose inhibition occurred in the metabolic sequence after the formation of glucose-6-phosphate, then 6-FG inhibition could be tentatively explained in terms of known pathways such as the hexose monophosphate path or the Emden-Meyerhof cycle. If either one of these known routes were involved, then one would expect to observe a greatly increased maximum inhibition of CO_2 formation with 6-FG if uniformly labeled glucose were replaced with C_1- or C_6-labeled glucose, depending on which of the two pathways was inhibited. However, since the maximum inhibition of radioactive CO_2 formation from C_1, C_6, and uniformly labeled glucose remains approximately the same, it appears that the inhibition imposed by 6-FG must be effected prior to the formation of glucose-6-phosphate. It may be that 6-FG interferes with a process concerned with cell permeation directly. This possibility was suggested by Blakley and Boyer,[9] who examined the effect of 6-FG on yeast fermentation

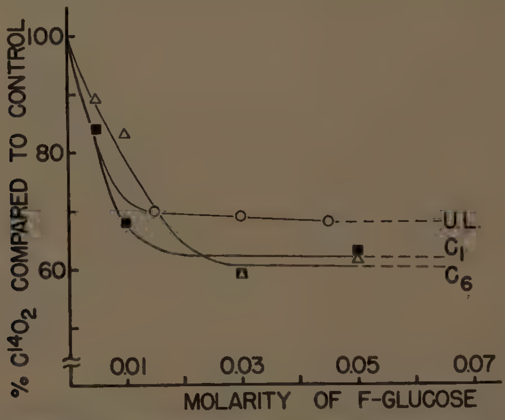

FIGURE 3. Comparison of the effect of 6-FG on the oxidation of C_1, C_6, and uniformly labeled glucose in kidney slices.

and concluded from their studies that the compound might be competing with glucose on the cell surface for cell entry. If 6-FG interference occurs at cell entry then, to be consistent with our data, two or more pathways of cell entry must be present. On the other hand, if 6-FG inhibits within the cell, then again, to be consistent with our data, a pathway alternate to that involving hexokinase must be present.

If one accepts the explanation for the plateau type of inhibition curve as suggesting that 6-FG inhibits a specific pathway for the oxidation of glucose to CO_2 and that an alternate unaffected pathway also exists, then one must conclude that the sensitive pathway is a common one in kidney slices, in adipose and diaphragm tissue of the rat, and in the extrahepatic tissues of the rabbit; but the extent to which the sensitive pathway operates in each tissue varies.

Summary

We have found that 6-FG inhibits the oxidation of glucose in rat kidney, and in diaphragm and adipose tissue, also that it inhibits the intracellular transfer of glucose and oxidation in the eviscerated-nephrectomized rabbit.

The inhibition of glucose oxidation by 6-FG is approximately the same regardless of the position of labeling in the glucose molecule. The fact that 6-FG produces an inhibition that does not exceed 30 to 40 per cent (kidney slices) when the inhibitor concentration is increased is interpreted to suggest that for glucose two or more pathways for metabolism prior to the formation of glucose-6-phosphate may exist. Alternatively, two or more methods of cell entry may exist for glucose.

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GLUCOSE TRANSPORT THEORY OF INSULIN ACTION

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About 10 years ago Rachmiel Levine, myself, and a number of colleagues began to direct our attention to the structure of the cell as a possible site of insulin action as opposed to a direct action upon one of the specific intracellular enzymes of intermediary metabolism.[1] In a surprisingly short period of time this approach to the mechanism of action of insulin achieved general interest. It is with considerable chagrin, however, that we must now view in retrospect earlier neglected statements of remarkable clarity that indicated the probability of facilitation of entry of sugar as the primary action of insulin.[2, 3] Such statements of Hober in 1914,[2] of Lundsgaard in 1939,[3] and of others passed with as little influence on the work of contemporary investigators as they did upon the continued investigations of their proponents. This was an era of all-consuming interest in the ever-developing field of enzymology. The search for the site of action of insulin paralleled the development of ideas of enzyme pathways. Each point of confluence of the flow of intermediary steps from protein, carbohydrate, and fat has briefly but temporarily occupied interest as a proposed point of insulin action.

In 1946 Soskin and Levine presented a critical review of this area of work[4] and gave expression to a growing general conviction that insulin must act at some very early enzymatic step. To designate the action of insulin, they described the hormone as promoting the entry of glucose into metabolism. The initial phosphorylation of glucose by hexokinase as the site of insulin action not only stood out as the most logical conclusion of all the past work, but also represented the last point of retreat through the enzyme schemes. When the subsequent demonstration of an action of insulin on hexokinase suffered the same fate of inacceptability on general testing, logical points of insulin-enzyme interaction were now exhausted, at least for the moment. A step ahead of intermediary metabolism was demanded, and this lay in the enveloping package for the enzymes of intermediary metabolism: namely, the limiting surface of the cell. We revived interest in the speculation of the relationship of cellular entry of sugar to the action of insulin by a new and independent demonstration. In essence, we were able to dissociate cellular entry of a glucoselike sugar from subsequent enzymatic or metabolic alterations of that sugar.[5]

We have reported experiments with the eviscerated-nephrectomized dog and rat in which insulin effected the area of distribution of glucose congeners.[5, 6] In this preparation, deprived of its abdominal viscera and kidneys, the remaining tissues comprise about 90 per cent of the body weight. Response to the presence or absence of insulin is retained with respect to glucose metabolism, but the capacity to metabolize a variety of other sugars is markedly altered. For many carbohydrate substances, the enzymes capable of chemical manipulation of these materials, being located exclusively in the abdominal viscera, are now absent. Such metabolically inert sugars, when introduced intravenously in the eviscerated-nephrectomized preparation, are distributed in whatever water compartments of the body are available to them. A family of hexoses and pentoses was found to occupy about 45 per cent of the carcass weight within 60 to 90 min. after intravenous administration. Beyond this point, any significant additional loss of these sugars from the blood and extracellular space where it was being measured was small and insignificant. The administration of insulin, either simultaneously with the sugar or at any point thereafter, promptly made available to a certain selected group of sugars an additional intracellular compartment, affording distribution of the material in total body water. This is best exemplified by FIGURES 1, 2, 3, and 4, where additional insulin serves to determine, not the rate metabolism of these inert sugars, but rather the area of distribution within the body of specific sugars.

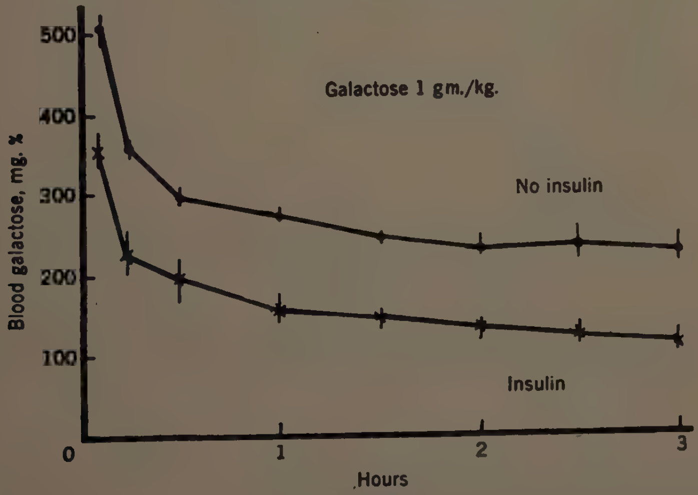

FIGURE 1. Data on eviscerated-nephrectomized dogs. Galactose injected at 0 time establishes a steady blood level representing the area of distribution of the nonmetabilized sugar. A spontaneous "galactose space" of 45 per cent is increased to total body water of about 65 per cent with added insulin. Reproduced by permission from *The American Journal of Physiology*.[5]

Of the sugars we employed, some proved to be insulin-responsive, while others were unchanged by the presence or absence of insulin. Those sugars whose intracellular penetration was promoted by the hormone revealed a common aspect of chemical structure in that they all resembled glucose in the configuration of hydrogens and hydroxyls about carbons 1, 2, and 3. The suggestion was made at that time that a barrier to the entry of sugars existed at the surface of certain cells, presumably skeletal muscle, heart, and adipose tissue. This barrier to entry was overcome by a specific system, located in a hypothetical cell-membrane structure, that transported glucose from the extracellular to the intracellular position. This glucose transport system, while being structurally designed for operation with glucose, would nevertheless accept and transport sugars of related structure sharing a common configuration about the first three carbons. Confirmation and extension of both these findings and their interpretation soon followed, and a direct demonstration for this analogous situation was finally made for glucose.[7-12]

Subsequent work has revealed that the specificity of this system is considerably wider in nature than experience with this early group of sugars had revealed. That the hexoses and pentoses possessing the same configurations

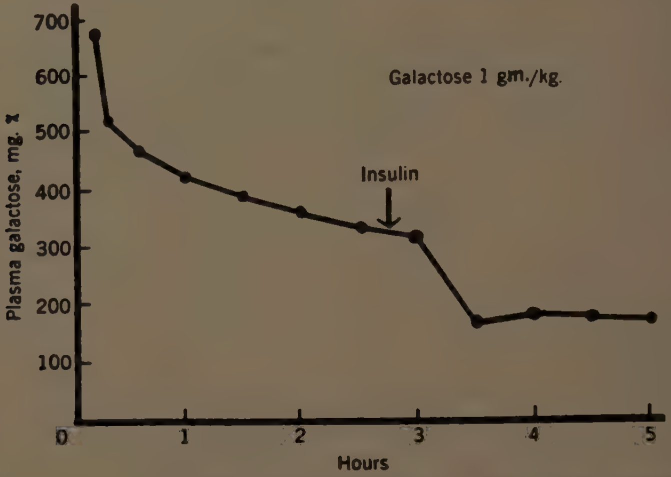

FIGURE 2. Data from eviscerated-nephrectomized dogs. The capacity of insulin to widen the area of distribution of glactose can be manifested at any time. Here, after the noninsulin level has been achieved, insulin added at 3 hours promptly makes the remaining intracellular water space available for greater distribution of the sugar. Reproduced by permission from *The American Journal of Physiology*.[5]

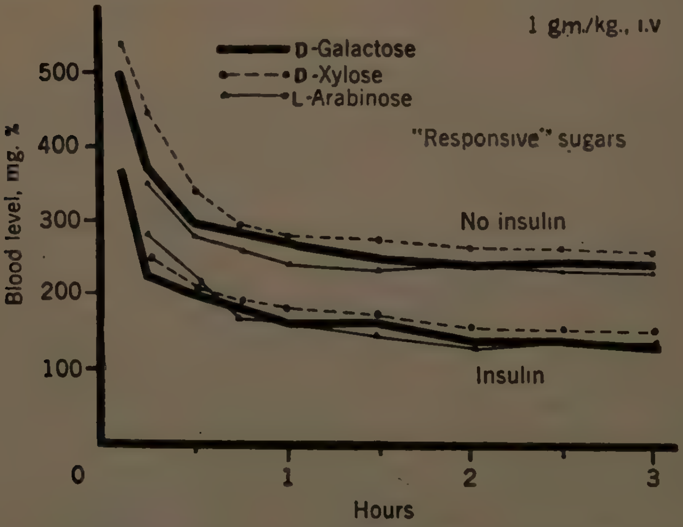

FIGURE 3. The capacity of insulin to increase the area of distribution is manifested on sugars other than galactose. Here D-xylose and L-arabinose respond to insulin in the eviscerated-nephrectomized dog, with increased entry into tissues, as seen by the lower blood levels. Reproduced by permission from *The American Journal of Physiology*.[6]

about carbons 1, 2, and 3 as seen in D-glucose makes them acceptable to this insulin-promoted sugar transport system remains unchallenged. Manipulations of the hydrogens and hydroxyls on carbons 2 and 3 indicate that the hydroxyl is not essential and can be replaced by a methyl group, hydrogen, or amine group. At least two sugars in which the configuration about carbon 1 and 2 are different from that of glucose, namely, mannose and fructose, have been reported as active participants in this transport system.[13-17]

These additional characterizations of the specificity of this system have in no way disproved the conception of a uniquely designed apparatus. In the same vein, some demonstrations of token action on other sugars are quite irrelevant.[18] The system appears to behave in a manner that is far from indiscriminate, and insulin can promote increased rates of entry consistent with movement of substrate amounts of carbohydrate for only a relatively few structured sugars.

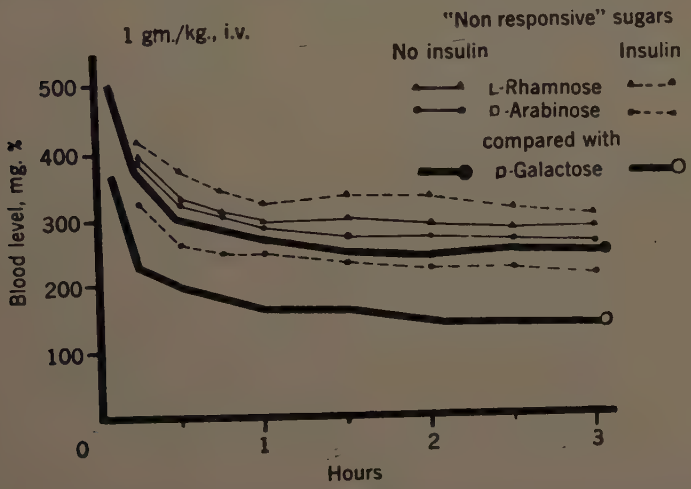

FIGURE 4. The sugar-transport action of insulin is quite specific, and not all sugars are affected. The distribution of D-arabinose and L-rhamnose in the eviscerated-nephrectomized dog is unaffected by added insulin. Reproduced by permission from *The American Journal of Physiology*.[6]

It would, indeed, be sad if we were now to compound our initial neglect of earlier speculative indications of cell structure as a site of action of insulin by neglecting to examine the vast literature dealing with specific transport systems for carbohydrates in other situations. Even a cursory examination of this area would indicate great differences between species and significant tissue specificities in transport systems for sugars in situations where insulin is without effect. Since much of the discussion of matters such as cell membrane and transport systems is largely a matter of conjecture, there is really no experimental basis for assuming that the stimulating action of insulin is directly involved with the intrinsic properties of this particular transport system itself. Specific transport systems for many substances, as well as for sugars, are rather universal properties of cells and tissues; indeed, there may be nothing intrinsically unique about this particular insulin-activated system. The finding that mannose or fructose is an acceptable sugar in the insulin system of the rat, but not in the carcass of the dog, may have no relation to either insulin action or to unrecognized differences in experimental technique. Cardiac muscle of the rat and skeletal muscle of the dog can readily be expected to display as much variation in carbohydrate detail as do the erythrocytes of these species. The rat,[9] the dog,[1] the rabbit,[13] the cat,[19] and man[20] have been employed by different investigators. The eviscerated carcass,[2] diaphragm,[15] gastrocnemius muscle,[16] heart,[12] aqueous chamber of the eye, and lens[11] have been used by various workers. Much has been made of occasional differences in the list of sugars affected by insulin without reference to species and tissue. There is general agreement in experience that insulin will promote cellular entry of a given discrete group of sugars and derivatives, and glucose is always included. These other materials are abnormal experimental tools and models for glucose.

There are other considerations that caution against simple identification of insulin with the actual apparatus of cellular transport of sugars. Insulin, with its own dependence on specific chemical structure for biological activity, is not the only agent that can activate the intracellular transport of a definitive group of carbohydrates. The capacity of contraction of skeletal muscles to increase glucose utilization in the diabetic organism has long been known. When insulin was assigned the role of relieving a barrier to the free entry of glucose as a limiting step in carbohydrate metabolism, it became apparent that increased glucose metabolism by the working animal could not be attributed to greater rates of disposal of intracellular metabolites. If the cellular entry of glucose was a limiting step in the first instance, it must also be so in the second. Since free glucose within the cell was essentially absent, greater intracellular utilization by work could not of itself lead to greater inflow of glucose. We were able to demonstrate in parallel experiments in eviscerated-nephrectomized animals that forced vigorous contraction of large masses of skeletal muscle would effect intracellular distribution of the same nonmetabolized sugars as are acted upon by insulin.[21] We proposed that the glucose transport system was activated not only by insulin but by some humoral factor that was released from strenuously contracting skeletal muscles. This demonstration could be achieved in the chronically depancreatized dog giving every indication of severe insulin deficiency. This phenomonon has similarly been confirmed by others, but it has been described as essentially a local and not a humoral effect of working musculature.[18] The action of skeletal muscle seems, indeed, to be a local effect with mild and moderate degrees of contraction. With severe contraction the effect becomes generalized, manifesting itself not only in the working muscle but in the resting tissues of the animal. More strikingly, demonstration of this humoral effect of working skeletal muscle can be achieved with the use of cross-transfusion techniques. Employing either an intact or an eviscerated-nephrectomized animal as the working partner, a resting, eviscerated-nephrectomized dog receives an exchange of venous blood by matched pumps in the order of 50 cc./min. and is disconnected from this vascular connection at the end of a 1-hour exposure to the circulation of the working animal. The resting animal, now free of any further contact with the working partner, will behave as if it were severely insulinized (FIGURE 5). Amounts of glucose that are adequate to maintain normoglycemia at rest prove inadequate and result in hypoglycemia. The amount of sugar required to maintain the normal blood level must now be doubled or even tripled. Furthermore, "insulin-responsive" sugars such as D-galactose or D-xylose will exhibit an area of distribution far wider than that seen in the noninsulinized resting state. The working partner can be chronically depancreatized and still yield an insulinlike effect. Neither the working partner nor the resting one has a source of insulin in this situation. Strenuous muscle work unquestionably yields some humoral influence that not only acts on the resting tissues of the working animal but, via cross-transfusion through plastic tubes, can influence an entirely resting preparation to exhibit augmented rates of intracellular transport of the same sugars that are acted upon by insulin.

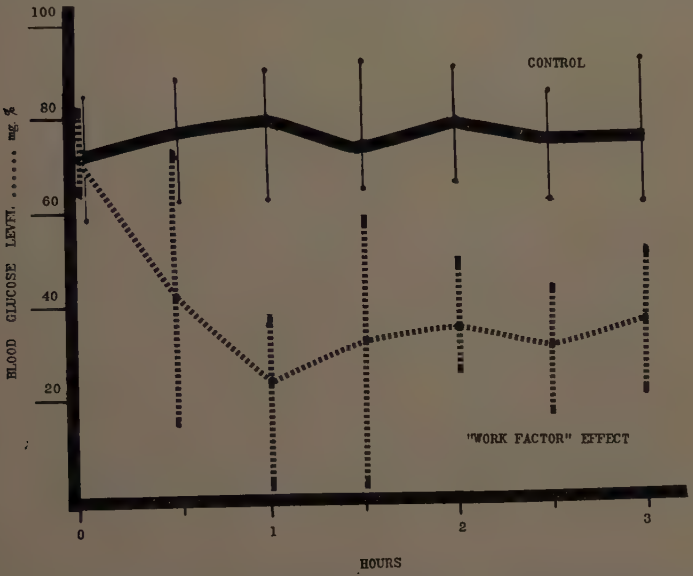

FIGURE 5. The presence of an insulinlike humoral factor arising from strenuous skeletal muscle work is demonstrated by cross-transfusion. These eviscerated-nephrectomized dogs were at rest throughout the experiment and were cross-transfused with either a resting (control) or working partner for 1 hour prior to testing. The working partner was either eviscerated or chronically depancreatized and was thus free of any source of insulin. The vascular connection was interrupted at zero time before testing the response of these resting recipient preparations. The glucose infusion (125 mg./kg./hour) is adequate to maintain normoglycemia in the control, but it is inadequate in the preparation that was previously exposed to the circulation of a working preparation, leading to hypoglycemia. The vertical bars represent the ranges seen in 6 animals in each group.

Of the many transport systems present in various tissues of the body, those related to glucose in skeletal muscle, cardiac muscle, and adipose tissue could be reasonably pictured as existing in some state of inhibition that is relieved by insulin and by some humoral factor released from strenuously exercising skeletal muscle itself. Although generally similar, the precise chemical configuration required by the transport system can be viewed as an intrinsic property of the specific tissue of a given animal. The activation or relief of inhibition of the system would be the area of concern of such things as insulin or the humoral factor of muscular work.

Such a normally inhibited state of the transport system would be consistent with the phenomona described by Randle and Smith[22, 23] concerning the relation of adequate oxidative metabolism of the diaphragm to a diminished rate of glucose entry. A variety of anoxic and chemical influences that will depress the generation of adequate high-energy-bond phosphates will lead to a freer rate of entry of glucose. This observation has been interpreted in terms of a direct involvement of adenosine triphosphate (ATP) in maintaining the state of some theoretical carrier material that effects the actual transport. It could equally be interpreted as the role of adequate oxidative metabolism and its available chemical energy for normal maintenance of some covering or inhibiting structure that blocks the expression of transport capacity. In functional or teleological terms, the extremely high capacity for glucose uptake that a completely insulinized skeletal muscle and adipose cell can exhibit is meaningful only in the immediate postabsorptive period. Exhibition of excessive amounts of insulin experimentally or pathologically in the fasting state leads to catastrophic hypoglycemia. These insulin-dependent sites of carbohydrate storage are faced with a dual functional problem; expression of rapid rates of glucose uptake in the brief postprandial period and severe inhibition of this high glucose-uptake capacity in the much longer fasting period. It would not be inconsistent with the physiological role of the sites of carbohydrate storage for inhibition of the transport system to be a major normal function of the biosynthetic activity of the cell.

Dissociating insulin action in our thinking from the intimate operation of sugar transport and seeking its influence upon relieving a normal inhibition of such a built-in apparatus might lend some resolution to a variety of growing demonstrations that insulin promotes the cellular uptake of at least certain amino acids.[24-26] In one of these approaches a nonutilizable amino acid is being used in a fashion analogous to the employment of galactose with reference to the glucose problems. Here, the entry of amino acids is dissociated from its subsequent metabolism either by degradation or by synthesis into protein. In other work the incorporation of radioactively labeled amino acids into tissue protein *in vitro* has been demonstrated to be augmented by insulin independently of any action on glucose. These are, indeed, independent data and have no direct bearing upon the data obtained with the carbohydrates. These are not alternative demonstrations, and they do not reveal some indiscriminate action of insulin in allowing all manner of materials to flush into the cell. Only certain amino acids share in this demonstration. Their quantitative aspects, including their relations to protein and to growth, remain to be examined. These are experiments involving very small amounts of materials in isolated *in vitro* situations. They are, nevertheless, well on the way to general confirmation and appear to be an easily reproducible demonstration of an action of insulin.

They could again be fitted into a scheme whereby insulin and such factors as muscle work, which parenthetically is itself a stimulus to protein synthesis, do not directly interact with transport systems themselves, but operate in some fashion to "uncover," or "reveal," or "allow" systems built into the surface of these cells to express themselves. For glucose, the secondary consequences of insulin's presence or absence are extremely apparent, leading to the syndrome of diabetes. The uptake of certain amino acids could very well be involved in a similar parallel fashion, but be obscured by both the relatively slow rate of amino acid turnover as compared with carbohydrates on the one hand and the very profound effects upon protein synthesis that are attendant to the metabolic consequences of impaired uptake of carbohydrate in diabetes mellitus.

The conviction that a cell surface site would explain the regulatory action of insulin on carbohydrate metabolism was derived from the behavior of a variety of sugars that served as models for glucose in response to insulin. The work of many others in these last few years has served only to strengthen the feeling that there is validity in constructing a picture of regulated rates of entry of materials at the surface of the cell as the site of action of insulin. There is no justification at this time, however, for any degree of rigidity in thinking about the details of such a system. Just as a glucose transport system is viewed as regulating the flow of substrate to the intracellular enzymes, the activity of the transport system in turn can be regulated by insulin by affording it access to the extracellular fluid that bathes it. The precise nature of the transport system itself may be a function of the particular tissue and the particular animal species, as indeed it is for analogous transport system in other tissues, where insulin is not involved. In similar fashion, if insulin serves to allow a transport system to express its innate properties by "uncovering" it in some fashion, it could afford the same opportunity to adjacent transport systems for such things as certain amino acids.

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REGULATION OF GLUCOSE UPTAKE IN HEART MUSCLE FROM NORMAL AND ALLOXAN-DIABETIC RATS: THE EFFECTS OF INSULIN, GROWTH HORMONE, CORTISONE, AND ANOXIA*

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Introduction

The regulation of glucose uptake in muscle tissue is recognized to be of great importance for the maintenance of a normal blood glucose concentration. A number of hormones, including insulin and secretions of the anterior pituitary and adrenal cortex, are known to be involved in this control. In addition, some nonendocrine factors such as muscular exercise and anoxia also play a role. In the present study we have attempted to make a detailed analysis of the process of glucose uptake with the object of obtaining a better understanding of how and at what point the above factors exert their effects. The various hormones and conditions that have been studied include the effect of insulin and anoxia on uptake by muscle from normal and diabetic animals, the effect of hypophysectomy and adrenalectomy on uptake by diabetic muscle, and the action of growth hormone and cortisone on uptake by muscle from hypophysectomized diabetic animals.

In order to analyze the action of the above factors it has been convenient to regard the process of glucose uptake as consisting of 3 sequential steps: first, the passage of glucose from within the capillary to the cell wall, second, the transport of glucose through the cell wall, and third, the phosphorylation of glucose inside the cell. The rate of uptake depends upon the resistance to the flow of glucose offered by each of these steps individually, but the step having the greatest resistance will be predominantly rate limiting.

In order to obtain satisfactory control of the experimental conditions we used an *in vitro* muscle preparation. The test object chosen was the isolated perfused rat heart. A technique was developed by which a small volume of bicarbonate-buffered medium at 37° C. containing glucose could be recirculated through the tissue by the normal vascular bed. The muscle has no cut edges, so all substances entering the cell must pass through the membrane. The viability of the preparation was excellent, as judged by its contractile activity and stable perfusion pressure. Details of the procedure will be presented elsewhere.[1]

Extracellular Transfer of Glucose

The question first examined was whether or not the first step in glucose uptake, that is, the passage of glucose between the perfusate and the cell, had an important rate-controlling function in glucose uptake. This step was determined indirectly by measuring the rate of extracellular distribution of sor-

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* The work reported in this paper was supported in part by grants from the Public Health Service, Bethesda, Md., the National Science Foundation, Washington, D. C., and the American Heart Association Inc., New York, N. Y.

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bitol, which is the nonmetabolized alcohol analogue of glucose and which cannot penetrate the cell. FIGURE 1 shows that sorbitol added to the medium equilibrated rapidly in a space corresponding to about 360 μl./gm. wet tissue. This space remained constant up to at least 50 min. of perfusion and was taken to indicate the volume of the extracellular water. This volume was unaffected by insulin, alloxan diabetes, or hypophysectomy.

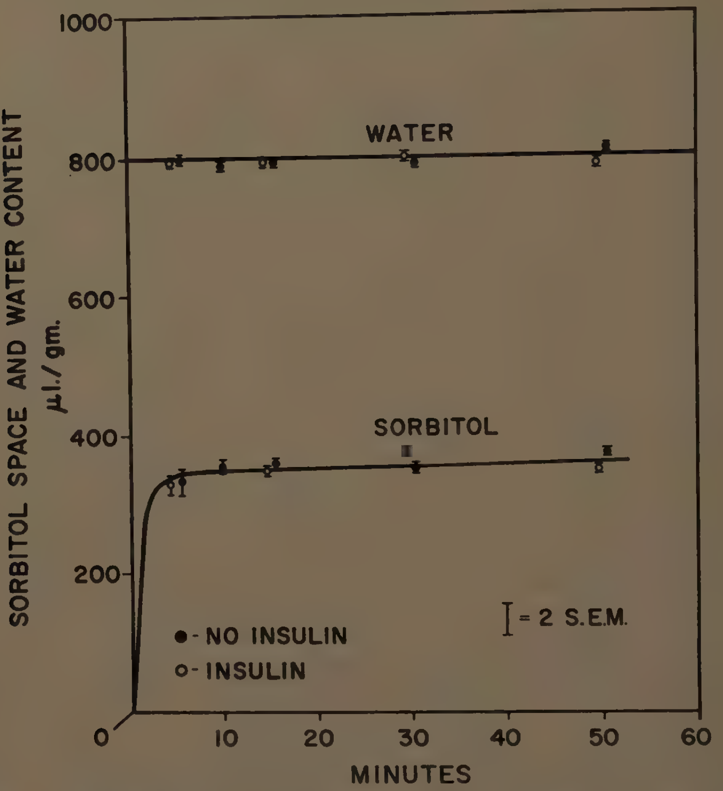

FIGURE 1. The sorbitol space and water content of the isolated perfused rat heart. Hearts were perfused with sorbitol-1-C^{14} (50 mg. per cent). Estimates of sorbitol in the perfusate and tissue extracts were based on radioactivity determinations. The distribution of sorbitol was the same in hearts from alloxan-diabetic or hypophysectomized rats as shown above.

The rate of sorbitol distribution in this space is indicated in FIGURE 2. The curve (*open circles*) shows that sorbitol left the capillary so rapidly that about 80 per cent of the extracellular water was equilibrated in 30 sec. The rate was not affected by insulin or by alloxan diabetes (*not shown*). It seems reasonable to assume that glucose equilibrates at least as rapidly as sorbitol, and this is suggested in fact by the glucose curves. The latter data, however, are difficult to evaluate in this connection, since glucose is not confined to the extracellular water, but enters the cell. From the sorbitol curve it could be calculated that the rate of transfer into the extracellular water was about 60 mg./gm./hr. at a perfusate concentration of 100 mg. per cent. This rate was 5 to 30 times faster than glucose entrance into the cell (*see below*); thus, the passage of glucose from the capillary to the cell cannot impose a major restriction on glucose uptake in this muscle under ordinary conditions.

Transport and Phosphorylation in Normal Heart Muscle

The control of uptake by steps 2 and 3, the transport and phosphorylation of glucose, was next considered. Earlier studies, reviewed elsewhere,[2] indicated that transport and phosphorylation were distinct from each other. Transport appeared to involve a specific, reversible chemical reaction of the sugar with a membrane component. This was shown by competition between glucose and a variety of sugars, including the common pentoses. The product of transport across the membrane was the free sugar, which was then phosphorylated by the hexokinase system inside the cell.

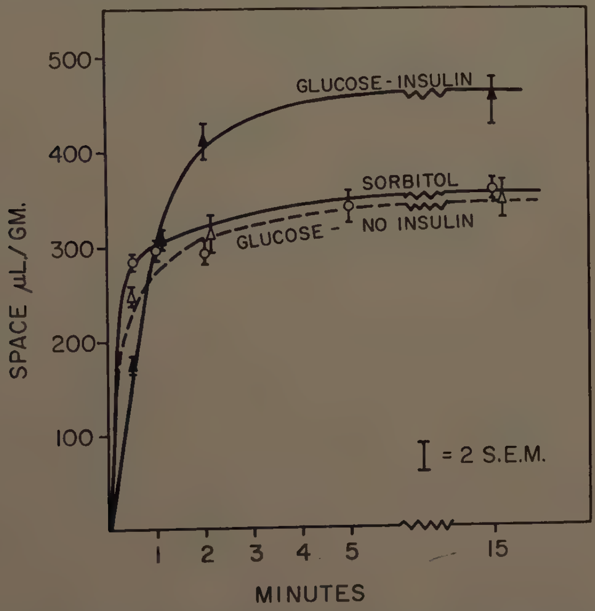

FIGURE 2. Time course of the equilibration of sorbitol and glucose between the perfusate and tissue water. Hearts were perfused with 50 mg. per cent sorbitol-1-C^{14} and 100 mg. per cent D-glucose. Insulin was added, as indicated, in a concentration of 3 μg./ml.

The rates of uptake and phosphorylation in hearts perfused at various concentrations of glucose with and without added insulin are shown in FIGURE 3. Uptake was determined by disappearance of glucose from the medium, and phosphorylation by the disappearance of free sugar from the medium and the tissue. The latter estimation was based on the assumption that all glucose disappearing must have been phosphorylated, since no phosphatase was present to regenerate free glucose.[3]

Uptake in the Absence of Added Insulin

The "no insulin" line in the upper graph of FIGURE 3 shows the values for glucose uptake and glucose phosphorylation, which were virtually identical in the absence of added insulin. It can be seen that the curve rose with increasing concentration and showed an obvious tendency to plateau at the higher concentrations. The corresponding curve in the lower graph for the free glucose space rose slightly, but then leveled off at a value not significantly differ-

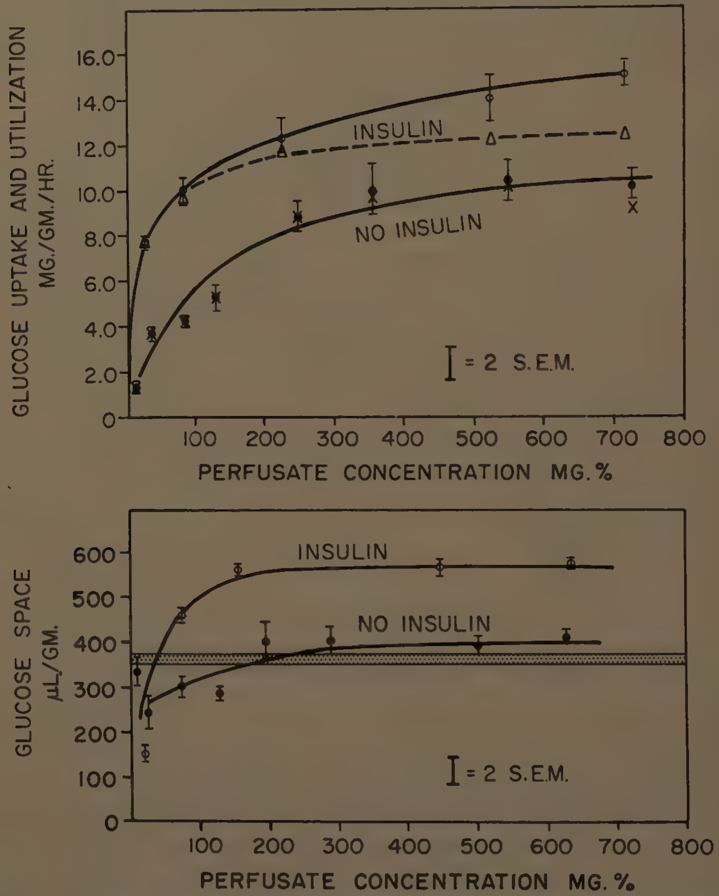

FIGURE 3. Glucose uptake and glucose space in normal hearts as a function of the glucose concentration of the perfusing medium. In the upper graph, the rate of uptake with insulin is shown by the open circles and solid line. The phosphorylation rate with insulin is shown by the broken line. The uptake rate without insulin (*indicated by closed circles*) is the same as the phosphorylation rate (*indicated by the symbol X*) and is represented by the lower solid line.

In the lower graph the extracellular space measured with sorbitol is shown by the stippled band, the width of which is 2 standard errors.

Hearts from 18-hour-fasted Sprague-Dawley rats were perfused for 30 min. in all cases. Insulin was added in a concentration of 3 μg./ml. Glucose determinations in these and all subsequent experiments were made by a glucose oxidase method.[4]

ent from the extracellular volume as measured by sorbitol; this is indicated by the crosshatched line. These data were interpreted as follows.

The uptake rose with increasing glucose concentration; hence the transport of glucose into the cell was accelerated. The transport, however, never became sufficiently rapid to exceed the capacity for intracellular phosphorylation, since no free glucose accumulated inside the cell, as shown by the fact that the glucose space did not exceed significantly the extracellular volume. Thus, phosphorylation could proceed only as fast as substrate was transported; that is to say, transport was the predominantly rate-limiting step over the entire range of glucose concentration.

Uptake in the Presence of Insulin

As shown by the uppermost lines of the upper graph, glucose uptake (*solid line*) and phosphorylation (*dashed line*) rose more rapidly with insulin, and phosphorylation reached a plateau at about 12 mg./gm./hr. Corresponding to this plateau, free glucose accumulated in large amounts inside the cell, as shown in the lower graph by the rise in glucose space far above the extracellular volume. The interpretation was as follows.

The uptake of glucose and consequently the transport of sugar into the cell were accelerated by insulin. The acceleration of transport was so great that, above 50 mg. per cent, the capacity for phosphorylation was exceeded, and free sugar accumulated inside the cell. The curves show that the insulin effect on uptake consisted of 2 components, both of which were secondary to transport acceleration. In the physiological range of glucose concentration the acceleration of transport led to a substantial increase in phosphorylation. At high glucose concentrations, however, phosphorylation was already near capacity in the absence of insulin. Acceleration of transport under these conditions caused only a slight and statistically insignificant further increase in phosphorylation, and the principal effect on glucose uptake was due to the accumulation of free sugar inside the cell. The data at high concentration indicated that insulin had little if any effect on phosphorylation that was not secondary to transport stimulation. It is also clear that, once the cell was saturated with free sugar, the rate of uptake was limited by the phosphorylation step.

Kinetics of Transport

Since uptake in the absence of insulin was limited by transport, the curve of uptake provided a picture of the kinetics of inward transport as a function of glucose concentration. The transport was unidirectional, since phosphorylation served as a trapping system for free sugar in the cell. It can be seen that the transport kinetics was clearly different from simple diffusion, where a linear relationship between rate and concentration would be expected. The curve, however, was well fitted by the Michaelis-Menten equations. FIGURE 4 shows a plot of the data by the Lineweaver-Burk method. The transport K_m, designated K_t on the graph, was about 110 mg. per cent glucose, that is 6×10^{-3} M, and the transport maximum was 12 mg./gm./hr.

Effect of Anoxia in Normal Hearts

Glucose uptake. Randle and Smith[5, 6] have shown recently that glucose uptake in muscle is accelerated by anoxia. This important observation has been studied further by Morgan *et al.*[7] Some representative data obtained with the perfused rat heart are shown in FIGURE 5.

The upper graph shows the rates of glucose uptake obtained when the medium contained 100 mg. per cent glucose; the corresponding glucose and sorbitol spaces appear in the lower graph. It can be seen that anaerobiosis markedly stimulated glucose uptake in the absence of insulin. Since transport was rate-limiting for uptake, it may be concluded that anoxia stimulated the transport step, as found earlier by Randle and Smith.[5, 6] The anoxia effect was like insulin in this regard, but differed in that no free glucose accumulated within the cell as seen with insulin administration. This absence of intracellular glucose suggested an increase in phosphorylation capacity induced by the anoxic state. This increase could be shown in the presence of insulin. Despite very high rates of uptake (hence rapid transport), no free sugar accumulated in the cell, and the limit of phosphorylation capacity was not reached. Under corresponding aerobic conditions, phosphorylation became limiting at a much lower level of uptake.

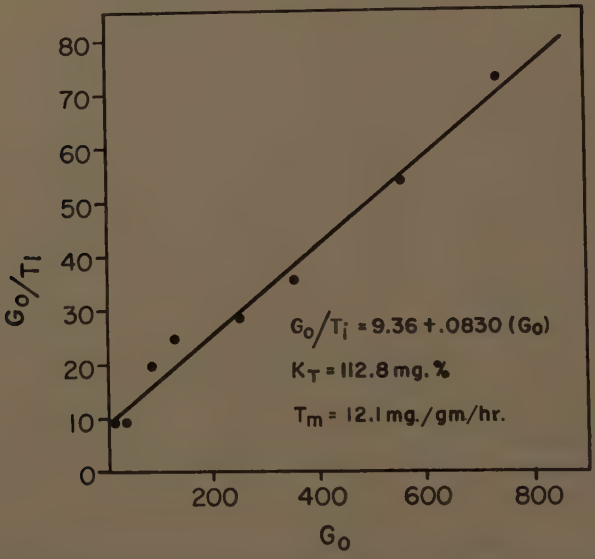

FIGURE 4. Glucose transport as a function of glucose concentration, plotted according to Lineweaver and Burk. The points have been calculated from the no-insulin data of FIGURE 3. The symbols are as follows: G_0, perfusate concentration; T_i, transport inward; K_T, concentration at which transport is half maximal; T_m, maximum possible transport rate.

L-Arabinose transport. The effect of anoxia on transport was also studied, using the glucose analogue L-arabinose. This pentose crosses the cell membrane by the same mechanism as glucose, as shown by competitive inhibition experiments, but is not detectably phosphorylated.[2] It was convenient to measure transport by determining the efflux of L-arabinose from the intracellular fluid of hearts that had accumulated a large amount of sugar during a preliminary perfusion. As shown in FIGURE 6, the rate of outward transport was a linear function of concentration under all conditions tested. In comparison to the control, anoxia caused approximately a twofold acceleration of trans-

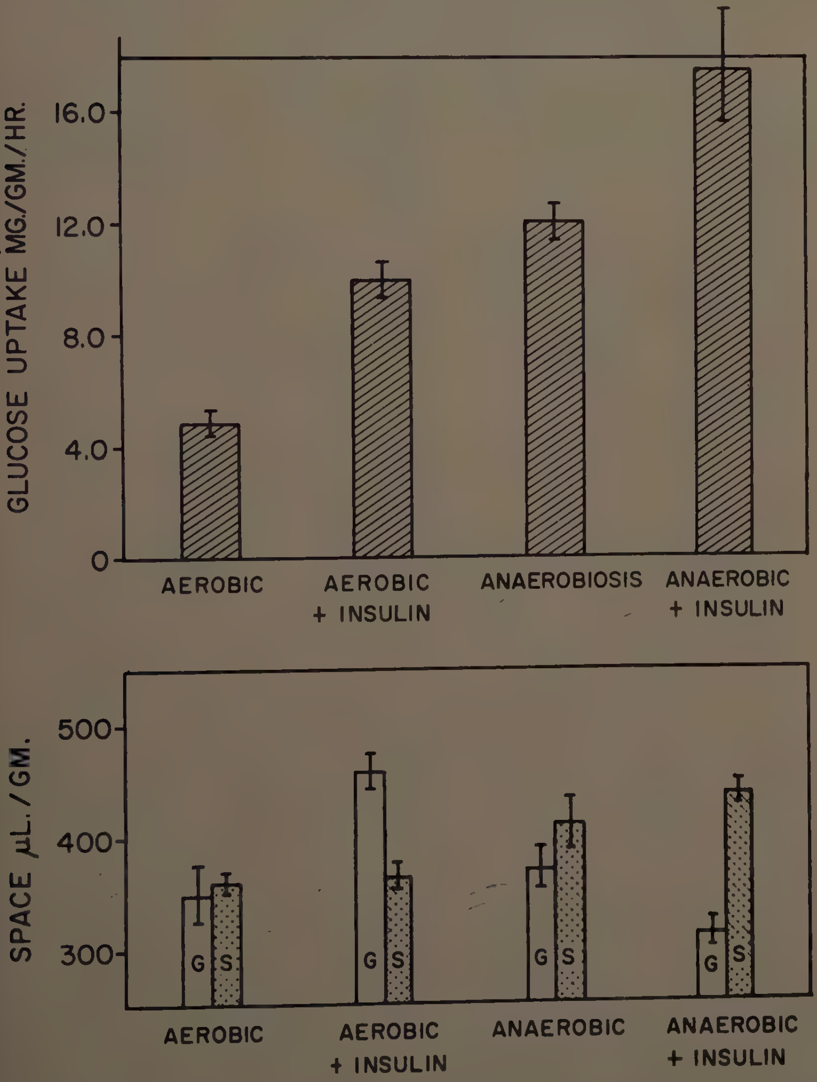

FIGURE 5. The effect of anoxia on glucose uptake and glucose space in the isolated, perfused heart from normal animals. In the lower panel the columns labeled *G* and *S* represent the glucose and sorbitol spaces, respectively.

The concentration of glucose was 100 mg. per cent and insulin 3 μg./ml. Perfusions were carried out at 37°C. for 30 min. Anoxia was induced by gassing the perfusion medium with 95 per cent N_2-5 per cent CO_2.

port. In the heart already treated with insulin, in which transport was accelerated about fourfold, anoxia had no additional effect. These data show that the transport effect of anoxia was much smaller than that of insulin. The two effects were not additive, as shown by the absence of an anaerobic effect in the fully insulinized tissue. It may be noted in passing that the data with L-arabinose provided a particularly clear demonstration of the effectiveness of insulin on transport under anaerobic condition.

Effect of Alloxan Diabetes on Glucose Uptake

Reduced rate of transport. The next series of experiments was concerned with the effect of alloxan diabetes on glucose uptake. The upper panel of FIGURE 7 shows that alloxan diabetes greatly reduced glucose uptake. Uptake was only slightly accelerated with the addition of insulin in the short time of these experiments. The glucose spaces are shown in the lower panel of FIGURE 7. The glucose space was less than extracellular in the diabetic, indicating that no free sugar was present inside the cell. It could be concluded, therefore, that transport was rate limiting for uptake in the diabetic and was much reduced. This confirmed our earlier observations in the whole animal[8, 9] and could also be demonstrated in experiments employing the nonmetabolized glucose analogue L-arabinose, the transport rate of which was found to be very low (see FIGURE 6).

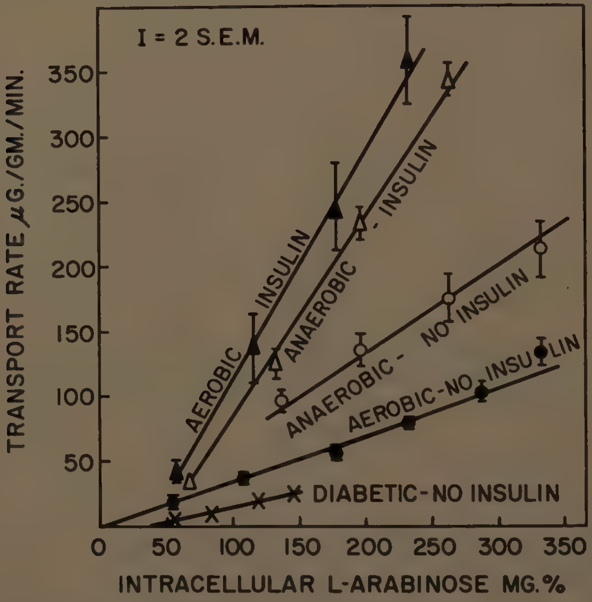

FIGURE 6. The effects of insulin, anoxia, and alloxan diabetes on the transport of L-arabinose out of the isolated perfused heart (see text for description).

Depression of phosphorylation. The addition of insulin to the diabetic heart stimulated transport into the cell, as shown by the accumulation of intracellular free glucose. Despite the abundance of sugar, however, the uptake of glucose rose very little. Since phosphorylation was clearly rate limiting under these conditions, it could be concluded that phosphorylation in the diabetic muscle was also impaired.

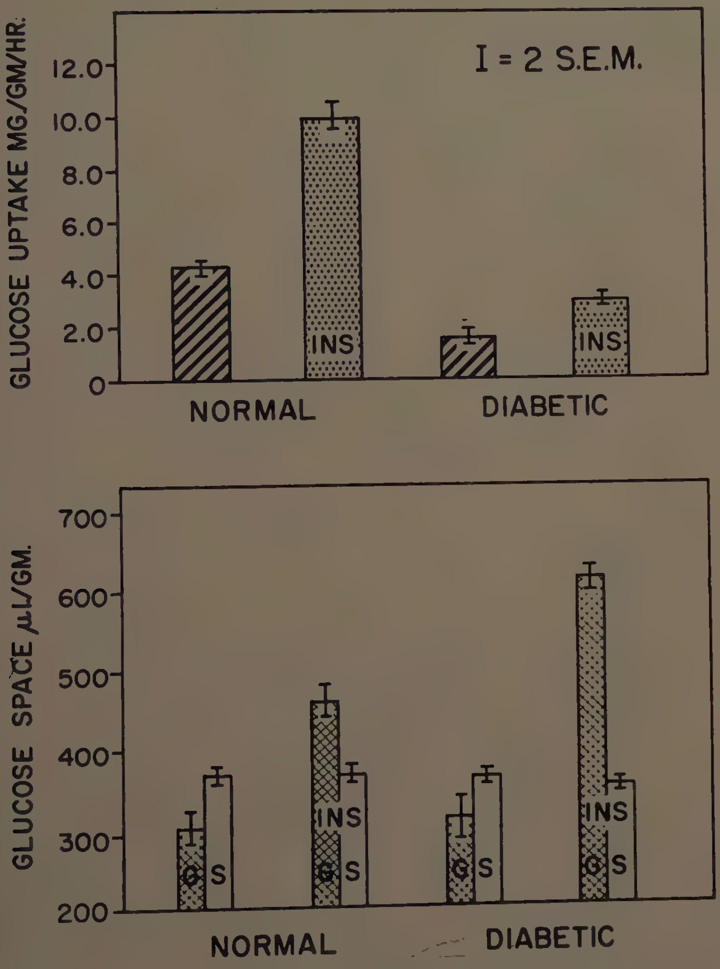

FIGURE 7. The effect of alloxan diabetes on glucose uptake and space in the isolated perfused heart. In the lower panel, the columns labeled *G* and *S* indicate the glucose and sorbitol spaces, respectively.

Perfusions were carried out for 30 min. with 100 mg. per cent glucose in the medium and, where indicated, 3 μg./ml. of insulin. Diabetic rats were prepared by the intravenous administration of alloxan (60 mg./kg.) 48 hours before sacrifice. Blood plasma glucose levels at the time of sacrifice averaged 700 mg. per cent after 18 hours of fasting.

It was also clear that insulin had no immediate major effect on the phosphorylation rate in diabetic muscle, in line with the result obtained with normal tissue.

Effect of anoxia. The effect of anaerobiosis on glucose uptake by diabetic hearts is shown in FIGURE 8. Glucose uptake was accelerated in the absence of insulin. Since transport was rate limiting, as shown by the absence of intracellular free sugar, anoxia must have increased the rate of transport. In the presence of insulin it is apparent that anoxia also increased phosphorylation very greatly, since uptake rose markedly without the accumulation of any free sugar in the cell. This increase was so great in fact that transport remained the rate-limiting step.

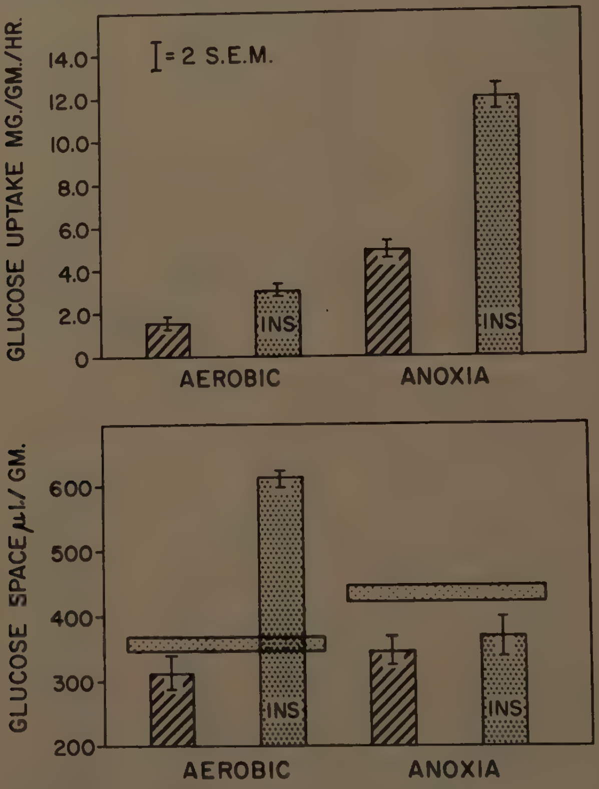

FIGURE 8. The effect of anoxia on glucose uptake and glucose space in the isolated perfused heart from alloxan-diabetic rats. The crosshatched line in the lower panel shows the extracellular (sorbitol) volume. The width of the line is 2 standard errors of the mean. The conditions were as described under FIGURES 5 and 7.

In other experiments it was found that a return to aerobic conditions after a period of anoxia resulted in the prompt reappearance of a depressed phosphorylation rate.

Effect of the Pituitary and Adrenal Secretion on Uptake by the Diabetic Muscle

The effect of pituitary and adrenal secretions on transport and phosphorylation in diabetic muscle were investigated in the experiments shown in FIGURE 9. Hypophysectomy in the diabetic caused no change in glucose uptake in the absence of insulin. Since transport was rate limiting under these conditions, it appeared that pituitary secretions did not have any striking effect on the transport rate. When insulin was added, the glucose uptake rose appreciably above that in the simple diabetic. Since phosphorylation was the limiting step, it could be concluded that removal of the pituitary resulted in an increased phosphorylation capacity. In other words, the pituitary secretion was in part responsible for the depressed phosphorylation of the diabetic muscle.

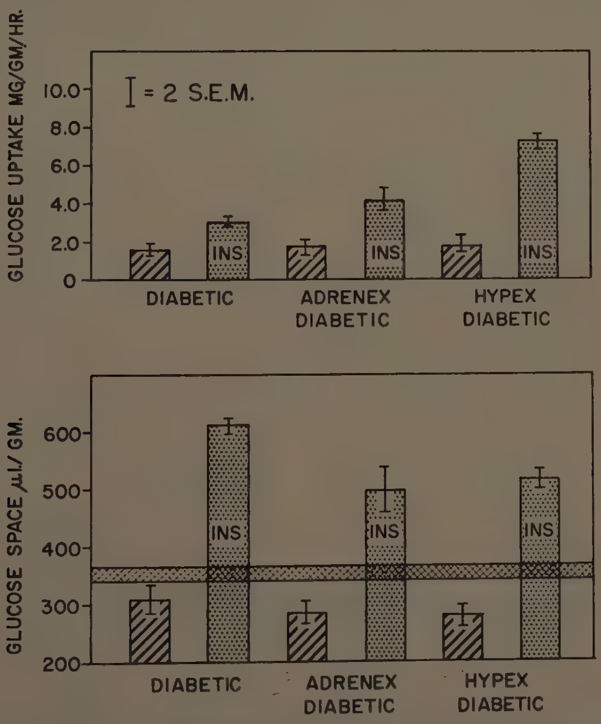

FIGURE 9. The effect of hypophysectomy and adrenalectomy on th.e glucose uptake and glucose space in the isolated perfused heart from alloxan-diabetic rats The crosshatched line in the lower panel shows the extracellular (sorbitol) volume.

The conditions were as described in FIGURE 7. Hypophysectomy was carried out 3 to 6 weeks prior to alloxan administration; adrenalectomy was carried out 24 hours before alloxan and 96 hours before sacrifice. Blood plasma glucose levels after 15 hours fasting averaged as follows: alloxan-diabetic, 700; hypophysectomized-diabetic, 320; adrenalectomized-diabetic, 450 mg. per cent.

The effect of hypophysectomy on the glucose uptake by the heart of normal rats was also investigated in some detail, and the results will be published shortly. It may be noted here that glucose uptake was appreciably lower than normal, and that this depression was due to a diminished rate of transport. Phosphorylation capacity in the presence of insulin was about the same as in the normal tissue.

The effect of adrenalectomy on the glucose uptake by the diabetic heart was not striking (see FIGURE 9). There was no rise in uptake in the absence of insulin and only a small rise in its presence. These data suggested that the adrenal secretions did not have any effect on transport, and that their inhibitory effect on phosphorylation was smaller under these conditions.

Growth Hormone and Cortisone

Since removal of the pituitary or adrenal appeared to improve the phosphorylation capacity in the diabetic muscle, the effect of growth hormone and/or cortisone readministration was studied, using hypophysectomized diabetic rats. These animals were treated either with growth hormone alone,

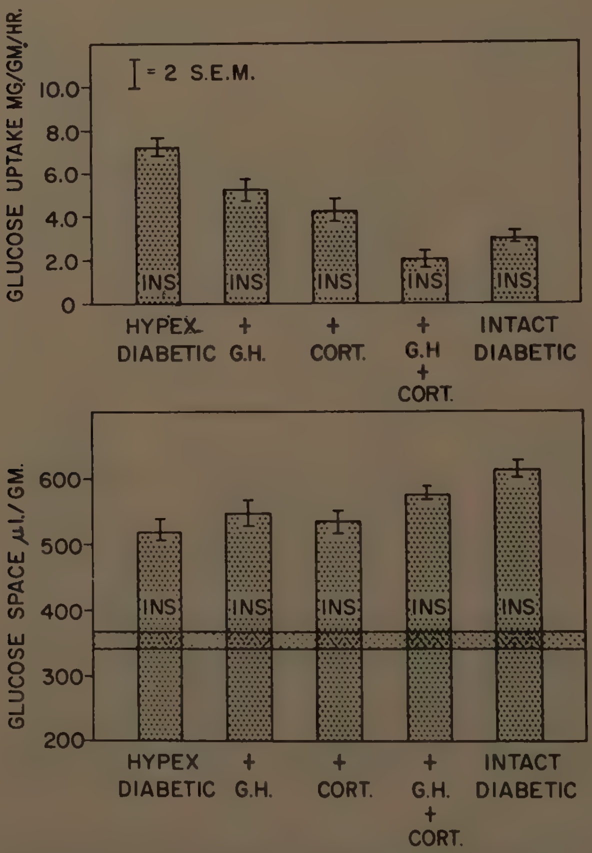

FIGURE 10. The effect of growth hormone and/or hydrocortisone on the glucose uptake and glucose space in perfused hearts from hypophysectomized alloxan-diabetic rats. The crosshatched line in the lower panel shows the volume of extracellular (sorbitol) space. The experimental conditions were as described in FIGURES 7 and 9. Bovine growth hormone (a gift from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md.) was injected 24 hours and again 12 hours prior to sacrifice in a dosage of 100 µg./100 gm. subcutaneously or intraperitoneally. Hydrocortisone was administered 24, 12, and 3 hours before sacrifice in a dosage of 2 mg./100 gm. At sacrifice, blood plasma glucose levels after 15 hours' fasting were as follows: no treatment, 320; growth hormone, 280; hydrocortisone, 700; growth hormone and hydrocortisone, 670 mg. per cent.

cortisone alone, or with both hormones together twenty-four hours before sacrifice. In all cases the hearts were perfused in the presence of insulin in order to establish a sufficiently rapid transport rate to accumulate intracellular free sugar and to make phosphorylation rate limiting for uptake.

The results are shown in FIGURE 10. Treatment with growth hormone alone led to a small depression of phosphorylation, as seen by the reduced uptake in the presence of abundant intracellular free glucose. Similarly, cortisone alone had a distinct inhibitory action. Both agents together were much more effective than either alone, and they depressed phosphorylation to the level of the simple diabetic.

Discussion

It was concluded from the present analysis of glucose uptake that the extracellular transfer of glucose from within the capillary to the cell was so rapid that the process did not have an important controlling influence on the overall rate of uptake. Some caution is necessary, however, in extending this conclusion, obtained with cardiac tissue, to other muscles, particularly resting skeletal muscle, where the circulatory system is largely shut down and is intrinsically sparser than in the heart. In fact, it would appear likely, as suggested earlier,[8] that accelerated transfer due to opening of the capillary bed may contribute to the increased glucose uptake by skeletal muscle with muscular exercise. It is also not clear at present whether or not extracellular transfer is affected by hormonal factors. Any such effects, if present, would be particularly important physiologically at low blood sugar concentrations, where the possibility is greatest that extracellular diffusion could become a rate-limiting step.

The studies with the heart preparation presumably reflect qualitatively the basic acceleratory action of insulin on the sugar transport process in muscle tissue in general, as first described by Levine and her associates.[10, 11] Our present work also shows the lack of any large, direct effect of insulin on phosphorylation. From a quantitative point of view, however, the insulin effect may be somewhat different in other muscles, particularly resting skeletal muscle. For example, it has been difficult to show accumulation of free sugar in the resting gastrocnemius with insulin[8, 9, 13, 14] under conditions in which it accumulates readily in the heart and diaphragm. This fact presumably reflects a different balance in the rates of extracellular transfer, membrane transport, and phosphorylation such that the transport rate, although accelerated by insulin, does not readily exceed the phosphorylation capacity.

The observation that glucose transport as a function of concentration follows Michaelis-Menten kinetics fits well with the concept that transport involves a chemical reaction between glucose and a membrane constituent, as reviewed elsewhere.[2] It is of interest that the K_m for transport in muscle is of similar magnitude to that found in the erythrocyte by LeFevre.[15] The K_m value is such that uptake is particularly sensitive to changes in blood glucose concentrations in the physiological range.

With regard to anoxia, the question of whether transport acceleration is due to a nonspecific loss of membrane integrity has been examined. The following four points, presented elsewhere in detail,[5-7] suggest that this is not the case: first, it has been shown that competition between sugars is preserved under anaerobic conditions; second, phloridzin strongly depresses transport and is as effective anaerobically as aerobically; third, anoxia does not accelerate transport in the insulinized muscle, as shown in FIGURE 6 (an effect would be expected if pores were created for diffusion); and fourth, the anoxia effect on transport and phosphorylation is promptly reversed with a return to aerobic conditions. In this connection we also found that the increase in sorbitol space with anoxia, seen in FIGURE 5, was returned promptly to normal by restoration of an oxygen atmosphere. This result indicated that the increase in sorbitol space was due to expansion of the extracellular volume and not to a leaky membrane.

The effects of anoxia are important in at least three respects: first, the effects on transport and phosphorylation may be of significance in increasing glucose uptake with exercise, where some degree of anoxia in the muscle is always present. Increased conversion of glucose to lactate under these conditions could provide additional adenosine triphosphate (ATP) without oxygen utilization at a time when the demand for ATP is very high and the supply of oxygen is inadequate. Anoxia in the diabetic muscle could also partially account for the well-known diminished insulin requirement of the diabetic individual with exercise. Second, the anoxia effect contributes to our understanding of the Pasteur effect, as pointed out by Randle and Smith.[5, 6] The acceleration of transport is particularly important in the case of muscle, since transport is the rate-limiting step for uptake in this tissue, as contrasted with most other tissues. Third, as discussed also by Randle and Smith,[5, 6] the effect of anoxia shows clearly that the rate of membrane transport is to some extent dependent on intracellular metabolic events. This observation suggests the possibility, of course, that insulin itself may act primarily within the cell, and that more rapid transport is secondary to intracellular metabolic changes, but whether or not this is the case has not been established. While insulin and anoxia both accelerate transport, it is clear that they differ in their actions in two important regards. The insulin effect on transport is much greater than that of anoxia (see FIGURE 6) and, conversely, anoxia strongly stimulates glucose phosphorylation, whereas insulin has little or no such immediate action.

The analysis of glucose uptake in the diabetic muscle shows that the first physiological defect is a reduced rate of transport; this result is in agreement with earlier studies in the whole animal.[8] The second defect, a depressed phosphorylation, was not appreciated in our earlier work, but has been suggested by the studies of Kipnis *et al.*[14] and is apparent in the present experiments. The relative importance of the transport and phosphorylation impairments can be analyzed as follows.

In the absence of insulin the depressed transport is most important, since transport is rate limiting. In the presence of insulin, however, when transport is accelerated the phosphorylation defect is most important, since phosphorylation becomes rate limiting under these conditions. The fact that insulin administration causes no acceleration of phosphorylation, at least in the short period of the present experiments, may account for some of the insensitivity to insulin observed frequently in severe diabetes with the initiation of treatment with the hormone. Presumably, the phosphorylation defect disappears with prolonged insulin treatment, but this has not been tested experimentally as yet in muscle.

The marked accelerating effect of anoxia, particularly in the presence of insulin, further emphasizes the importance of the depressed phosphorylation in diabetic muscle. In view of the high rates obtained with anoxia, it is clear that the phosphorylation impairment is not due to a deficiency of enzyme. This is in contrast to studies in diabetic liver, where marked alterations in enzyme levels have been found.

The contribution of the pituitary and adrenal glands to the metabolic defect in diabetes fits in general with the concept suggested by Levine and Goldstein[16] and first supported experimentally by the studies of Kipnis, which are reported elsewhere in this monograph. According to this proposal, pituitary and adrenal factors affect the phosphorylation step, and insulin effects are confined to transport. In agreement with this view, hypophysectomy or adrenalectomy does not improve the depressed transport in the diabetic, suggesting that the block at this point is due simply to the absence of insulin. This block remains rate limiting for uptake until insulin is given. Transport acceleration then shifts the rate-limiting step to the phosphorylation level. Adrenalectomy of the diabetic raises phosphorylation capacity slightly, and hypophysectomy has a marked effect on it. This increase in capacity makes room for a much larger effect of insulin on the glucose uptake. Injection of growth hormone and/or cortisone in the hypophysectomized diabetic depresses phosphorylation. Either hormone alone has some effect, but the largest response is obtained with both together. This observation is in accord with many studies showing a synergism of these hormones in inducing a diabetic state.

Summary

The process of glucose uptake has been analyzed in the perfused isolated rat heart. The extracellular transfer of glucose is very rapid and does not limit glucose uptake in the normal or diabetic tissue. The transport of sugar through the membrane is the rate-limiting step in normal and diabetic hearts. The rate is reduced in the diabetic and is not accelerated by hypophysectomy or adrenalectomy. Transport is stimulated, on the other hand, by anoxia in both the normal and diabetic muscle. At the same time anoxia markedly increases the phosphorylation capacity. Insulin stimulates transport to a much greater extent than anoxia, but does not immediately increase phosphorylation capacity to any great extent. In the presence of insulin, transport may be accelerated sufficiently so that phosphorylation becomes the rate-limiting step for uptake. Under these conditions it has been shown that phosphorylation is depressed in the diabetic muscle. This depression is partially relieved by adrenalectomy and largely abolished by hypophysectomy. The depression of phosphorylation is partially restored by either growth hormone or hydrocortisone administration and is fully re-established when both hormones are given together to the hypophysectomized diabetic rat. Thus, the regulatory mechanisms for trans-

port appear to be insulin and the level of aerobic metabolism. Factors regulating phosphorylation are pituitary (growth hormone) and adrenal (hydrocortisone) secretions and the presence or absence of aerobic metabolism.

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EFFECTS OF INSULIN ON ADIPOSE TISSUE*

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The purpose of this report is to summarize observations made in the Baker Clinic Research Laboratory on the action of insulin on adipose tissue. We have become impressed by the sensitivity of this tissue to insulin, as well as by the magnitude of the response obtained. Indeed, it appears to us that adipose tissue may well represent the major site of insulin action in the intact organism. This reappraisal of the metabolic importance of adipose tissue is also in keeping with the extensive studies carried out in other laboratories, notably those of Wertheimer and Shapiro,[1] Hausberger,[2] Favarger,[3] and Stetten.[4] In general, it may be stated that the role assigned to this tissue is rapidly changing from that of an inert storage site to that of an extremely active and labile system intimately concerned with the synthesis, storage, oxidation, and release of body fats.

In the early studies, adipose tissue was shown to respond to insulin by an accelerated glucose uptake,[5] by increased glycogen synthesis both *in vivo*[6,7] and *in vitro*,[8] by a marked elevation of the respiratory quotient to values greater than unity[9] and, in the hands of some investigators, by an increased oxygen consumption.[10] More recently the use of carbon-14 and deuterium-labeled compounds has provided a more quantitative appreciation of the insulin effect. FIGURE 1 summarizes the average of several experiments with glucose randomly labeled with carbon-14. The marked insulin effect is easily seen, namely, a sixfold increase in the oxidation of glucose to CO_2 and an approximately tenfold increase in fatty acid synthesis. Glycerol synthesis is doubled and glycogen synthesis is increased almost twentyfold, although these metabolic fates are quantitatively less important in the total disposition of glucose carbon.

Since added insulin produces such a marked stimulatory effect, adipose tissue from animals that are presumably insulin deficient should exhibit a decreased metabolism of glucose. FIGURE 2 compares the disposition of labeled glucose in normal and alloxan-diabetic rats, with and without insulin added to the incubation flask. The failure of insulin *in vitro* to elevate the decreased metabolism of adipose tissue from the diabetic animal to that of the normal one treated with insulin may be due to other secondary or adaptive phenomena; however, the markedly decreased basal level of glucose metabolism in adipose tissue from the diabetic organism is evident.

Milstein[11] first reported a much greater increase in the oxidation of the first

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* The work reported in this paper was supported in part by Grant A-2460 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and by a grant from the Adler Foundation, Inc., Rye, N. Y.

† Recipient of a Research Fellowship from the Dazian Foundation, New York, N. Y.

‡ Recipient of a Fellowship from the Governments of the Dominion of Canada and the Province of Quebec.

carbon (carbon-1) of glucose when compared to the last carbon (carbon-6) after insulin stimulation. It was therefore postulated that insulin selectively stimulated the pathway responsible for the preferential oxidation of glucose carbon-1, namely, the phosphogluconate-oxidative pathway. However, subsequent experiments by Winegrad[12] in our laboratory, including recovery of carbon-14 in fatty acids, demonstrated that with or without insulin stimulation twice as much carbon-6 as carbon-1 was recovered in fatty acids, as shown in FIGURE 3. This observation suggested that about one half of the fatty acids synthesized from glucose are derived through a pathway whereby carbon-1 is lost and the remainder through a pathway whereby carbon-1 is not lost. Again, this was true either with or without insulin stimulation. From these

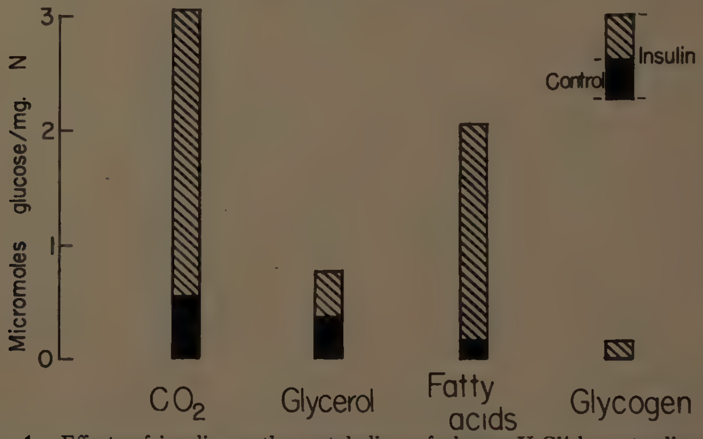

FIGURE 1. Effects of insulin on the metabolism of glucose-U-C14 by rat adipose tissue. The insulin concentration is 0.1 U./ml.

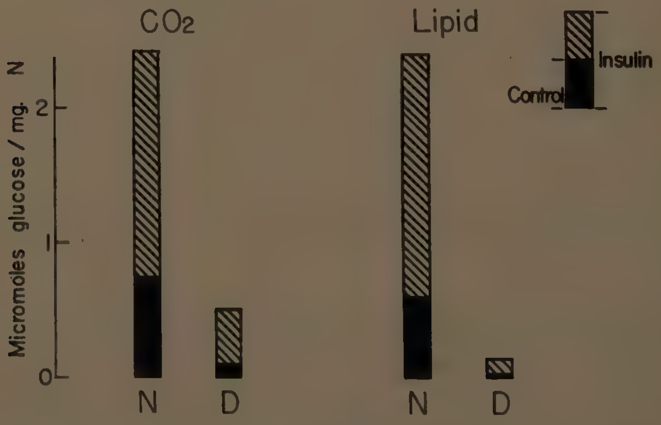

FIGURE 2. Effects of insulin on the metabolism of glucose-U-C14 by adipose tissue from normal (N) and from alloxan-diabetic (D) rats. The insulin concentration is 0.1 U./ml.

data it would appear that glucose is metabolized equally by two pathways, and that insulin stimulates both pathways to the same extent or, more probably, that insulin stimulates the conversion of medium glucose to intracellular glucose-6-phosphate, the metabolic precursor from which both the glycolytic (or Emden-Meyerhof) and the phosphogluconate-oxidative pathways diverge.

Acetate is also utilized by adipose tissue for fatty acid synthesis, as are many other substrates. In FIGURE 4 further evidence is presented to indicate that the insulin effect appears to be mediated primarily by increased availability of glucose to intracellular metabolism.[12] Adipose tissue was incubated with acetate-1-C^{14}, and the incorporation of label into lipid and CO_2 was measured with and without added insulin and with and without added glucose. No effect is observed with insulin alone. Glucose alone doubles acetate incorporation into lipid, while glucose and insulin together increase the incorporation of acetate into lipid by a factor of 10.

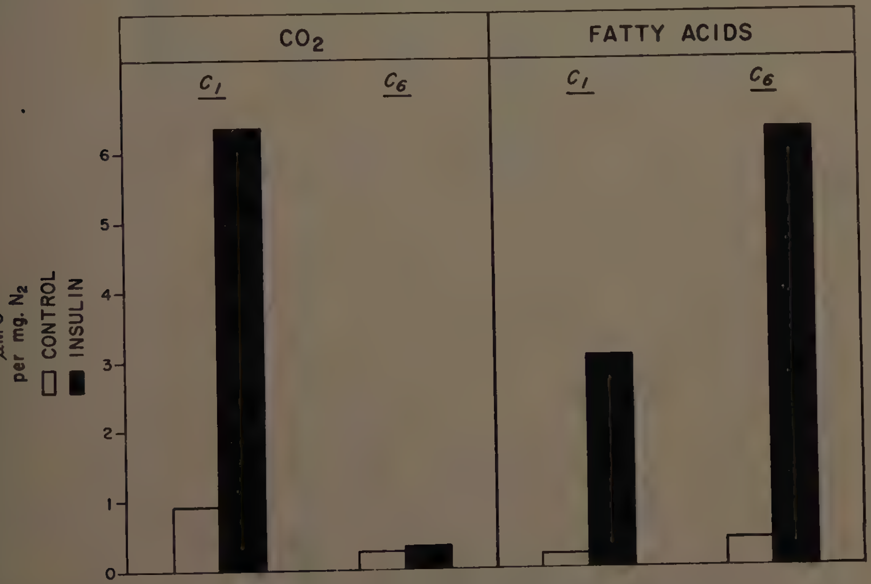

FIGURE 3. Effects of insulin on the metabolism of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue *in vitro*. Bicarbonate buffer, 3-hour incubation, 20 mM/l. glucose, 0.1 U. insulin. Reproduced by permission from *The Journal of Biological Chemistry*.[12]

If this insulin effect, as the previous data have suggested, were mediated by an increased conversion of medium or circulating glucose into tissue glucose-6-phosphate (note that our system does not differentiate between a process involving increased permeability or translocation and one involving phosphorylation), a similar pattern of glucose metabolism should be obtained whether the increase is secondary to insulin or secondary to increased glucose concentration in the medium. Recent experiments were performed whereby the concentration of glucose and the concentration of insulin were varied independently.[13] FIGURE 5 demonstrates the relation of glucose-1-C^{14} and glu-

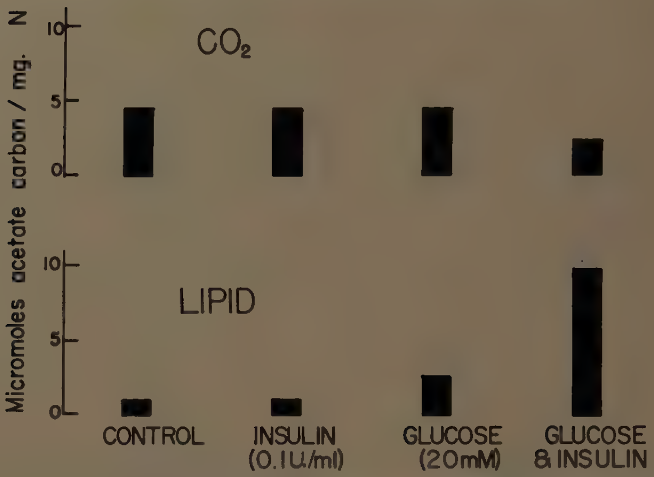

FIGURE 4. Effects of glucose alone, insulin alone, and glucose plus insulin on the metabolism of acetate-1-C^{14} by adipose tissue.

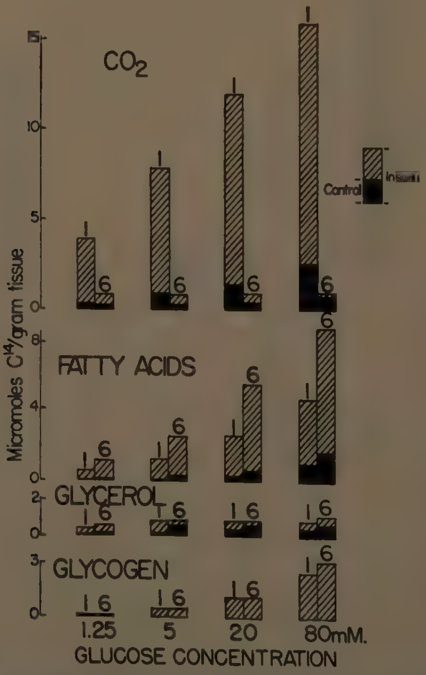

FIGURE 5. Effects of varying concentrations of glucose on the metabolism of glucose-1-C^{14} and glucose-6-C^{14} by adipose tissue with and without added insulin (0.1 U./ml.). The numerals 1 and 6 at the top of each column refer to the specifically labeled glucose used.

cose-6-C^{14} metabolism to varying concentrations of glucose in the medium. Again, the C-1/C-6 ratio in fatty acids remained constant at about 0.5. Comparison of the oxidation of glucose-carbon-1 with that of carbon-6 reveals that the increment is mainly from carbon-1. FIGURE 6 reveals an entirely similar pattern, although in these experiments the concentration of glucose was maintained constant at 20 mM/l. (360 mg. per cent), whereas insulin concentration was varied from 0 to 100,000 μU./ml. The similarity of the two patterns shown in FIGURES 5 and 6 clearly suggests that the increased metabolism induced by increasing concentrations of glucose is identical to that induced by insulin action. This is further support for a unifocal hypothesis of insulin action resulting primarily in facilitation of the conversion of extracellular glucose to intracellular glucose-6-phosphate.

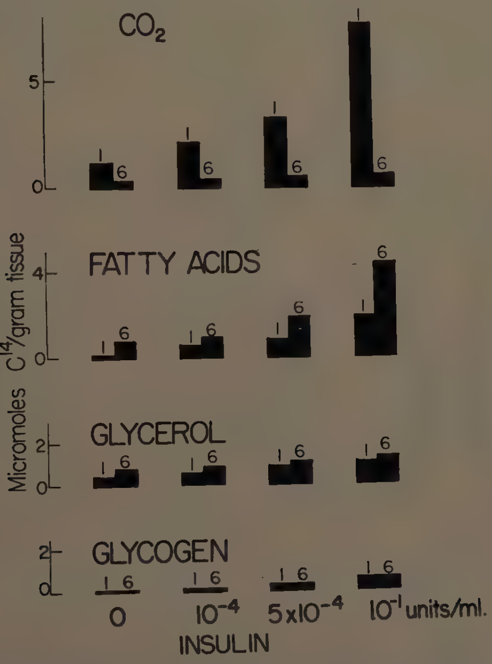

FIGURE 6. Effect of varying concentrations of insulin on the metabolism of glucose-1-C and glucose-6-C^{14} by adipose tissue. The concentration of glucose is 20 mM/l. throughout

In tissues such as muscle, it is now well established that insulin increases the uptake and/or metabolism of other sugars similar in structure to glucose. Adipose tissue has been incubated with labeled fructose, galactose, or mannose. All three sugars appeared to undergo oxidation in this tissue and also to be incorporated into tissue lipids. Furthermore, all three were found to be insulin-sensitive. Mannose was metabolized as actively as glucose; fructose and galactose less so (approximately one fifth and one twentieth the rate of glucose, respectively).

Recent and preliminary experiments by Y. Dagenais and G. Zahnd in our laboratory suggest an interesting effect of potassium on glucose metabolism by adipose tissue. In the total absence of this cation (FIGURE 7) glucose oxidation, fatty acid synthesis, and glycogen synthesis are all greater than in its presence. However, when insulin was added, potassium augmented the insulin effect. At present, one can only speculate as to the mechanism and significance of these findings.

The synthesized fatty acids are deposited in the tissue; only a trace is released into the medium even in the presence of albumin as an acceptor. Partition of the lipids in the tissue itself shows that 98 per cent or more of the labeled fatty acids are present as esters. However, incubation with labeled fatty acids such as palmitate reveals the process of rapid exchange of tissue fatty acids with those in the medium.[14] In addition, if the medium fatty acid concentration is raised above 0.7 mEq./l., the tissue shows a net fatty acid uptake in addition to still greater exchange of tissue fatty acids with those in the medium.

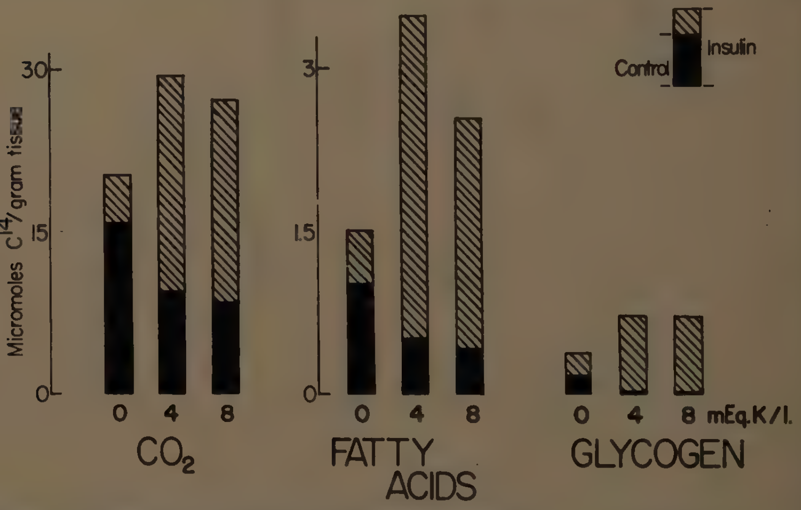

FIGURE 7. Effects of varying concentrations of potassium on the metabolism of glucose-1-C^{14} by adipose tissue with and without added insulin (0.1 U./ml.).

The glycerol used to esterify the fatty acids is synthesized *de novo* from glucose. Other experiments have shown, in confirmation of those of Shapiro *et al.*,[15] that added labeled glycerol is utilized to a very limited degree. In the control tissue, the synthesis of glycerol is 17 times greater, mole for mole, than is the rate of synthesis of labeled fatty acids. In the presence of insulin, the relative turnover of glycerol is less, but still 3 times that of the fatty acids. This turnover is more evidence for the great metabolic activity of what has long been considered an inert tissue.

Recently, growth hormone[16, 17] and epinephrine[18] have also been shown to increase glucose uptake by normal adipose tissue *in vitro*. The patterns of metabolism resulting from the presence of these two hormones and also that resulting from the addition of ACTH[19] differ markedly from that due to insulin. In all three instances there appears to be a proportionately greater increase in glycerol turnover, a markedly increased oxidation of carbon-6 (compared to carbon-1), decreased lipogenesis and, as is well known, an outpouring of unesterified fatty acids from the tissue into the medium. In the case of epinephrine, as in the case of insulin, studies with glucose labeled in either the 1, 2, or 6 positions have permitted an evaluation of the relative activities of several metabolic pathways. Schematically (FIGURE 8), when one compares the metabolism of insulin-stimulated tissues to that of control tissues, there is a simultaneous increase in both the glycolytic and the phosphogluconate-oxidative pathways, as stated previously, with predominant metabolism of glucose carbon to fatty acids and to CO_2, the latter being derived from the phosphogluconate-oxidative pathway, as well as from the decarboxylation of pyruvate to acetate. The increased uptake after epinephrine, on the other hand, is associated primarily with increased glycolytic activity and glyceride-glycerol synthesis. Fatty acid synthesis is much less and, of the small quantity synthesized, much is released into the medium along with active release of unlabeled unesterified fatty acids. Recent experiments have also shown a simultaneous outpouring of formaldehydogenic substance (presumably glycerol) from the tissue. Insulin and epinephrine added together increase the glucose uptake to an extent greater than that resulting from either alone, but the resulting metabolic pattern is identical to that produced by epinephrine alone. That epinephrine leads to marked glycogenolysis in adipose tissue obtained from fasted-refed animals has recently been established both *in vivo* and *in vitro* and by chemical as well as by histochemical techniques.[20] In the case of growth hormone, a specific stimulation of the uronic acid pathway has been suggested as the best explanation for the specific increase in the oxidation of carbon-6 by adipose tissue.[17] On the other hand, the *in vitro* addition of hydrocortisone (25 μg./ml.) to adipose tissue[21] does not alter either its baseline

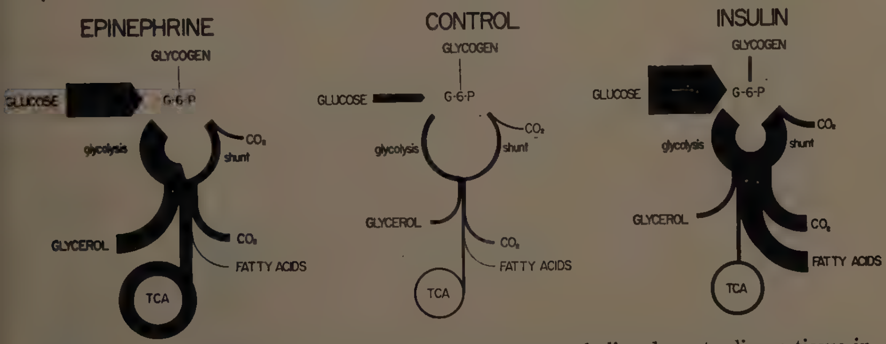

FIGURE 8. Diagrammatic representation of glucose metabolism by rat adipose tissue incubated without the addition of hormones and in the presence of either insulin (0.1 U./ml.) or epinephrine (0.1 mM/l.). The thickness of the bars indicates the relative rates of metabolism. The data upon which this diagram is based were derived from several studies.

metabolism or the effect of insulin on lipogenesis from glucose labeled in carbon-6, as shown in FIGURE 9.

Finally, it should be stressed that the effect of insulin on adipose tissue is obtained with concentrations well within the physiological range. In fact, if one accepts present estimates of the concentration of insulin in normal human serum as approximately 100 μU./ml., it is noteworthy that alterations in the metabolism of glucose by adipose tissue *in vitro* are obtained with one tenth of this physiological concentration of the hormone. This sensitivity has led us to employ adipose tissue incubated in the presence of glucose-1-C^{14} for the purpose of measuring insulinlike activity in serum.[22]

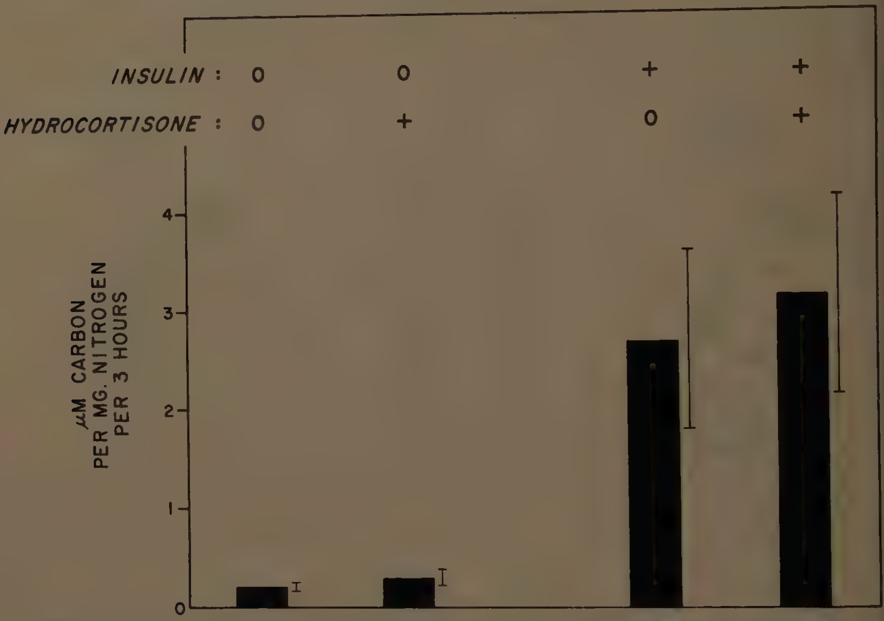

FIGURE 9. The effect of hydrocortisone added *in vitro* (25 μg./ml.) on lipogenesis from glucose-6-C^{14} by rat adipose tissue.

In conclusion, it should be re-emphasized that the marked sensitivity of adipose tissue to insulin, as well as its extreme vascularity and relatively great bulk, suggests that adipose tissue may well be a major, if not *the* major, site of insulin action when insulin is present in physiological amounts in the intact organism.

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EFFECTS OF INSULIN AND TOLBUTAMIDE ON BLOOD GLUCOSE ENTRY AND REMOVAL RATES*

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During the past several years we have been conducting experiments, using C^{14}-labeled glucose, with the object of obtaining information concerning the factors involved in the regulation of entry and removal of the blood glucose. As a result of these studies[1,2] we were led to the conclusion that insulin exerts its hypoglycemic action not only by accelerating the removal of glucose from the blood, but also by suppressing hepatic glucose output. These studies left in doubt the quantitative importance of this hepatic action of insulin, but indicated that under certain circumstances it might be of major significance in blood sugar regulation. We shall describe briefly the principles of our procedures and present certain experiments that lead us to believe that insulin suppresses the hepatic output of glucose.

The procedure used represents an extension of a familiar isotope-tracer technique employed previously for a study of glucose turnover in rats, dogs, and man.[1,2] A trace dose of glucose uniformly labeled with C^{14} is injected, and blood samples are removed at frequent intervals for determination of blood sugar content and specific activity.

No attempt is made in this brief report to describe the details of our methods of determination of specific activities and of calculations; all of these have been published. An experiment illustrative of many thus far conducted in normal postabsorptive human subjects is shown in FIGURE 1, which illustrates the typical behavior of the blood glucose level and specific activity in a normal 16-hour-fasted human.

There is thus a rather precise balance between entry and removal of the blood glucose, the rates of which can easily be estimated from the first-order reaction-rate expression.

When insulin is injected, the blood sugar falls, then returns to normal. This hypoglycemic effect must be due either to a suppression of entry or to an enhancement in removal, or to both. If the hypoglycemia is due to an acceleration of removal, the specific activity will continue to fall at least as rapidly as before, since the incoming glucose molecules are diluting a pool that continuously becomes smaller; however, if the drop is due in some part to an inhibition of entry, the specific activity will fall less rapidly, since the blood glucose will be more slowly diluted by new, unlabeled molecules. The behavior of the specific activity curve when hepatic glucose output ceases may be seen in FIGURE 2. In this experiment a normal dog was submitted to hepatectomy after a portal caval anastomosis. During the period of the operation the specific activity of the blood glucose dropped exponentially but "plateaued"

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* The work reported in this paper was supported in part by grants from the Public Health Service, Bethesda, Md., The American Cancer Society, Inc., New York, N. Y., and the United States Atomic Energy Commission, Washington, D. C.

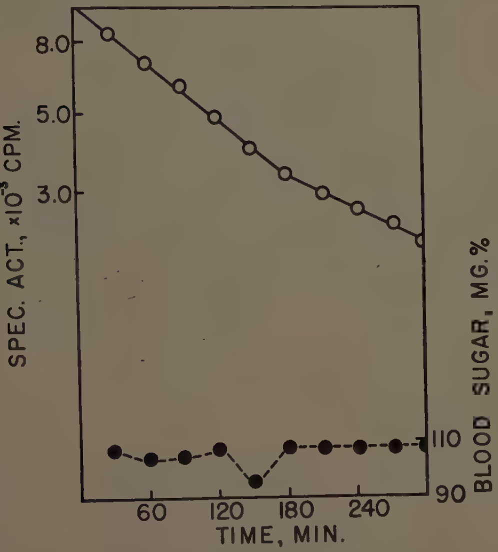

FIGURE 1. Blood glucose turnover and oxidation in the normal fasting human. During the 5-hour period during which the blood sugar level was constant (*bottom*) there was an exponential drop in specific activity shown in the upper curve. Although there is some tendency to decrease with time, the general pattern is a constant decay of radioactivity accompanied by a relatively constant blood sugar level.

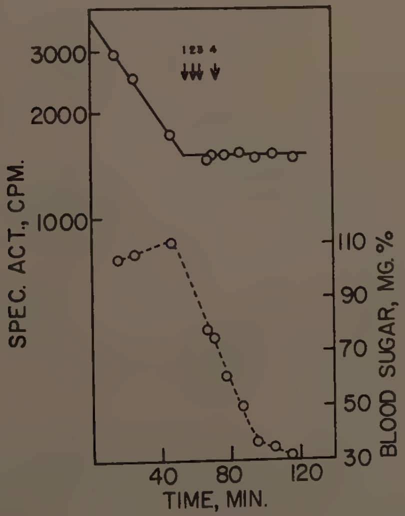

FIGURE 2. Blood sugar levels and specific radioactivity in a normal dog after a 1-stage hepatectomy performed essentially after the procedure of Firor and Stinson.[3] Radioactive glucose was administered at zero time. Arrows 1, 2, and 3 respectively indicate the ligation of the portal vein, the hepatic artery, and the inferior vena cava. The fourth arrow indicates the removal of the liver. The hepatectomies were performed by Aaron Bannett of the Albert Einstein Medical Center.

when the hepatic circulation was occluded and remained constant until the dog died with a blood sugar level of 30 mg./100 ml.

The type of evidence that points to an hepatic action of insulin is shown in FIGURE 3. This experiment was conducted with a fasted, normal human, a resident of a local home for the aged. Exactly similar results were obtained with trained normal dogs that were unanesthetized, unrestrained, and fasted for 24 hours. A trace dose of highly radioactive C^{14}-labeled glucose was injected at zero time. At intervals thereafter blood sugar samples were collected and, at the time indicated by the arrow, insulin was injected intravenously in a dosage of 1 u./kg. At short intervals thereafter, samples of blood were again removed for determination of the level and specific radioactivity. In this experiment, which is illustrative of many thus far carried out, there is an exponential drop in the blood sugar specific activity preceding the insulin injection, indicating a constant rate of blood glucose turnover. Immediately after insulin injection there is consistently observed a plateauing in the specific activity; during a short interval ranging from 10 to 20 min. following intravenous insulin injection, and always exactly coinciding with the period of maximum drop in the blood sugar level, the specific activity remains constant. At the time when the blood sugar stops dropping and begins the upward trend, the specific activity again resumes its downward trend. We interpret these findings as indicating that, during this immediate postinsulin period, unlabeled glucose molecules cease to enter the blood stream. Since the liver is generally recognized to be the only organ contributing glucose to the blood, we

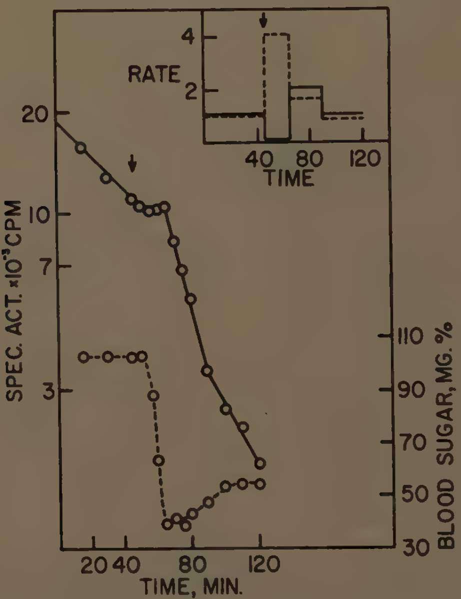

FIGURE 3. Hepatic action of insulin in a fasted normal human. See text.

assume that this effect is brought about by a suppression of hepatic glucose output. However, the method is not designed to locate the source of the inflowing glucose, but only its occurrence.

From the data thus obtained we can estimate rough approximations of inflow and outflow rates during these various intervals preceding and following insulin administration;[1] these are shown diagrammatically in the inset of FIGURE 3, where the solid line denotes entry and the broken line denotes removal of the blood glucose. No claims are made as to the accuracy of these estimations. In view of the large number of assumptions required in such calculations, we regard these only as indicative of the orders of magnitude over the time sequence studied. Three phases are recognized: first, prior to insulin injection there is a constant rate of turnover in the range of about 1 mg./100 ml./min.; second, immediately after insulin injection the rate of entry drops essentially to zero, while the removal rate increases about threefold; and, in the third phase, entry recommences and the removal rate drops, each to approach the preinsulin values.

These results, which point to an immediate action of insulin in suppressing hepatic glucose output, are in agreement with those of Bearn *et al.*,[4] who found, using hepatic catheterization, that in both normal and diabetic patients insulin caused an immediate suppression of glucose output and even a reversal of flow in some instances. In experiments similar in principle to ours, Berson and Yalow,[5] Searle *et al.*,[6] Henderson *et al.*[7] and Wall *et al.*,[8] also observed diminution of hepatic glucose output following insulin injection, but the latter two groups did not regard this effect as important in its hypoglycemic action. On the other hand, Ashmore *et al.*[9] failed to observe plateaus in similar experiments in rats, and though they did observe such plateaus in experiments with dogs, they were unable to confirm a suppression of hepatic glucose output by portal-hepatic sampling techniques (personal communication from J. Ashmore). Tarding and Schambye,[10] using both tracer methods and portal-hepatic sampling, found a suppressive action of tolbutamide, but not of insulin, on hepatic glucose output.

It is now appropriate to discuss our studies with the oral hypoglycemic agent, tolbutamide. There is already much evidence to show that this, as well as similar agents, act by suppressing hepatic glucose output,[5,6, 9-13] rather than by increasing glucose utilization, but there is also a large body of evidence indicating that they require insulin for their action.[14,15] FIGURE 4 illustrates our results with tolbutamide in a normal human. Just as with insulin, there was a plateauing in specific activity following its intravenous injection, which coincided with the induction of hypoglycemia and terminated at the low point in blood glucose level. This finding is readily explainable in terms of an insulinogenic action of tolbutamide, with a consequent suppression of hepatic glucose output. However, when estimations are made of glucose entry and removal rates from these data we find, as shown in the insert, that there is suppression of entry, but essentially no increased removal.

These findings, which suggested an apparently exclusive action of tolbutamide on hepatic glucose release, seemed at first glance to be at variance with an insulinogenic action of the drug. However, the possibility was envisioned that insulin, when secreted in response to tolbutamide, exerted its major effect on the liver. To simulate a slow, regular introduction of insulin into the blood, insulin was given in approximate doses of 0.1 u./kg. by subcutaneous injection; otherwise, the experiments were conducted exactly as were the previous ones. Under these conditions two features are noteworthy, as shown in FIGURE 5. First, we observed an extremely long plateau in specific activity, lasting as

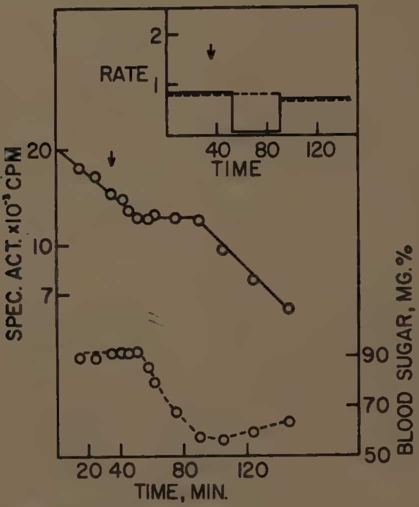

FIGURE 4. Effect of tolbutamide in the normal human. See text.

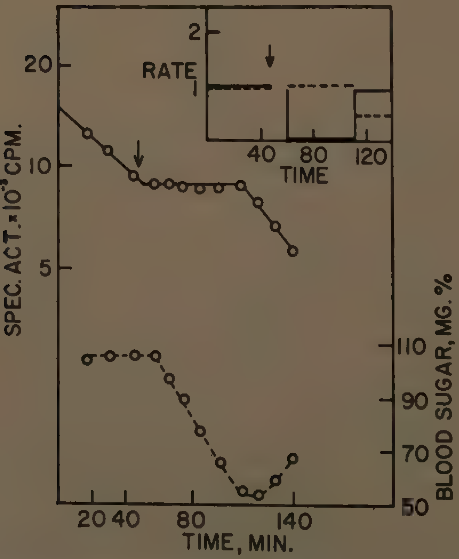

FIGURE 5. Action of subcutaneous insulin on the release of hepatic glucose. See text.

long as 1 hour, in contrast to 10 to 20 min. when injected intravenously in a single dose. Second, as shown in the inset, the suppression of hepatic glucose output occurs apparently without an increased peripheral utilization. Other investigators also have observed recently that the slow introduction of insulin may result in hepatic effects without peripheral effects; these investigators include, notably, Craig *et al.*,[16,17] Dulin and Johnston,[18] and Madison and Unger.[19]

In a very recent paper Berson *et al.*[20] pointed out that our values for initial glucose turnover may be too high, owing to incomplete mixing of the labeled glucose with the body pool during the preinsulin equilibration period. For this reason the lack of a peripheral effect may be fortuitous. We ourselves have to recognize the justice of this criticism. In long-term turnover experiments we avoided the uncertainty of incomplete mixing and have indeed obtained somewhat lower initial fasting turnover rates in humans. The results of 7 such recent experiments are shown in TABLE 1. Preinsulin turnover rates are shown in column 2, and in columns 3 and 4 are given the rates of inflow and outflow calculated over the period, usually about 1 hour, during which the blood sugar fell to its lowest point. The values for turnover ranged from 0.5 to 1.4 mg./100 ml./min. in the series of experiments carried out in 5 normal and in 2 mildly diabetic humans in which at least 80 min. were allowed for equilibration before insulin injection. In the first 3 experiments there was no appreciable change in glucose removal rate, while entry rate during the period of insulin injection dropped to zero in 2, and to 0.3 in the third. In 2 experiments the removal rate increased somewhat, and in one of these the entry dropped, but did not stop entirely. In the 2 diabetics, who were very mild cases, the blood glucose removal rate did not increase, and the the entry dropped to zero.

TABLE 1

EFFECT OF SUBCUTANEOUS INSULIN INJECTION ON BLOOD GLUCOSE ENTRY AND REMOVAL IN MAN

(Values are in mg./100 ml. blood/min.)

Condition	Insulin dose μ/kg.	Preinsulin turnover	Insulin, entry	1-hour removal
Nondiabetic	0.18	0.87	0	0.9
	0.14	1.0	0	1.0
	0.15	0.90	0.3	0.9
	0.10	0.95	0.7	1.2
	0.11	0.5	0.2	0.9
Diabetic	0.13	1.4	0	0.9
	0.10	0.7	0	0.6

We wish to emphasize something that we have repeatedly stressed in the past. We do not regard these replacement rates as anything but rough approximations. What we regard as of significance is the plateauing of the specific activity curves, which tell us that glucose is not entering the blood and, in turn, allow us to estimate that the blood sugar drop is not due to a markedly increased removal rate.

Without denying an important role to insulin in promoting the utilization of glucose by peripheral tissues, we feel that the data here presented, together with those of previous and contemporary investigators using a variety of experimental techniques, provide sufficient grounds for the belief that insulin exerts an immediate, probably direct effect on glucose release by the liver. All of the investigators who have used the isotope tracer procedure have admitted some hepatic action of insulin. It happens that our experiments indicate to us that the hepatic effect is consistent and that, under certain circumstances, it may have been dominant in the resulting hypoglycemia. The same situation presumably exists among the investigators who have been using portal and hepatic blood-sampling techniques for determination of hepatic insulin effects. Each laboratory uses techniques that differ in some respects from those of all of the others. Under the circumstances it is probably overoptimistic to expect better agreement in dealing with the action of a substance such as insulin, whose action on the liver has been controversial ever since its discovery.

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INFLUENCES OF GLUCOSE LOADING AND OF INJECTED INSULIN ON HEPATIC GLUCOSE OUTPUT*

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Whether insulin acts immediately on the liver to reduce its rate of glucose output is a matter of great current interest. From the theoretical aspect, the answer given to this question has serious implications with respect to hypotheses concerning the mechanism of insulin action. In particular, an increase in permeability to glucose of the hepatic cell membrane should increase rather than decrease its rate of glucose output. In the hepatic cell, in contrast to the muscle cell, the net flow of glucose is from inside to outside the cell, and an increased facility for glucose transport across the cell membrane of the kind postulated for insulin action on the muscle cell could serve only to increase this flow and not to decrease it.

Several investigative groups have used C^{14} glucose in attempts to supply the answer to this question, and the divergent findings that have resulted have been summarized and evaluated in a recent article.[1] In the present communication special consideration is given to the extent to which these divergencies are due, on the one hand, to differences in experimental findings brought about by differences in experimental conditions and procedures, and are due, on the other hand, to differences in interpretation and calculation.

The Body Glucose Pool

If the glucose pool were as we should like to define it rather than as it actually is, it would consist of glucose in aqueous solution in a well-stirred space (the solid lines of FIGURE 1) of constant volume. New glucose molecules entering the space would mix instantly with all of the glucose already present, and no carbon atom of any glucose molecule leaving the space would ever reappear in the space as a part of a glucose molecule. If to this idealized structure is added the physiological information (1) that C^{12} glucose in fact is continually being added to the pool, and (2) that prior to insulin injection the same number of molecules of glucose is in fact continually being removed from the pool so that the concentration of glucose in the aqueous solution remains unchanged, relatively speaking, one has the basic model used by all groups of investigators who have studied the influence of insulin on hepatic glucose output.

In FIGURE 1 the letter V represents the unchanging volume of plasma and associated tissue fluids that contain the glucose pool. Inasmuch as V is assumed to remain constant, the total grams of glucose in the pool (designated B_t) at any time can be calculated from the glucose concentration measured in a plasma sample withdrawn for analysis. What is required for this calculation is knowledge of how much glucose there is in the pool at any one given value of glucose concentration; this, in effect, is the same as knowing the value of V. Wrenshall and Hetenyi,[2] after performing repeated single injections of C^{14} glucose, have concluded that the value of V does not change to an important extent during insulin action. Of the other letters appearing in FIGURE 1, A_t represents the total number of microcuries of C^{14} glucose in the pool at time t, R_1, and R_2 the amount (grams) of glucose carbon per minute entering and leaving the pool at time t, and F the amount of C^{14} glucose ("weightless" amount) in microcuries per minute being given by constant infusion (F is zero in those experiments called "single injection" experiments). The specific activity of the glucose in the compartment may be designated i_t and is equal to the ratio A_t/B_t when the specific activity is expressed as μc. C^{14} per gram of glucose carbon.

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* The work reported in this paper was carried out at the Brookhaven National Laboratory under the auspices of the United States Atomic Energy Commission, Washington, D. C.

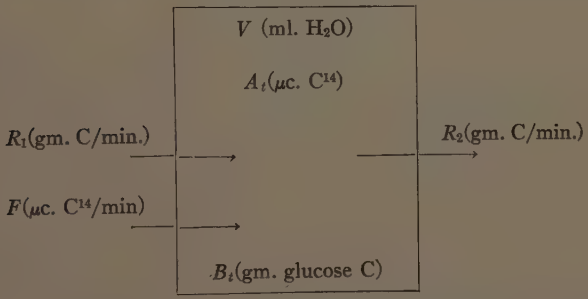

FIGURE 1. A diagram representing the conventional model for the dog body glucose pool. For explanation of the symbols see text.

$$\text{Since } A_t = B_t i_t$$

$$\frac{dA_t}{dt} = B_t \frac{di_t}{dt} + i_t \frac{dB_t}{dt} \tag{1}$$

Also, it can be seen by inspection that the rate of change of the total glucose present in the compartment depends on the two flow rates R_1 and R_2 :

$$\frac{dB_t}{dt} = R_1 - R_2 \tag{2}$$

Similarly, it can be seen by inspection that the rate of change of the total C^{14} present in the pool depends on F (the rate of C^{14} infusion) and on the product of R_2 (outflow rate) and $i_{(t)}$ (the specific activity of the glucose in the compartment):

$$\frac{dA_t}{dt} = F - R_2 i_t \tag{3}$$

Combining EQUATIONS 1, 2, and 3, there are obtained equations for R_1 and R_2 :

$$R_1 = \frac{F - B_t \dfrac{di_t}{dt}}{i_t} \tag{4}$$

$$R_2 = R_1 - \frac{dB_t}{dt} \tag{5}$$

For numerical approximations these equations can be used in the forms:*

$$R_1 = \frac{F - \left(\dfrac{B_{(t)} + B_{(t+\Delta t)}}{2}\right)\left(\dfrac{i_{(t+\Delta t)} - i_{(t)}}{\Delta t}\right)}{\dfrac{i_{(t)} + i_{(t+\Delta t)}}{2}} \tag{4a}$$

$$R_2 = R_1 - \frac{B_{(t+\Delta t)} - B_{(t)}}{\Delta t} \tag{5a}$$

When F is zero as in the "single-injection" procedure, integrating EQUATION 5 then combining it with EQUATION 4 and again integrating gives:

$$R_1 = \frac{(B_o - B_t) \ln \dfrac{i_o}{i_t}}{t \ln \dfrac{B_o}{B_t}} \tag{4b}$$

$$R_2 = R_1 + \frac{B_o - B_t}{t} \tag{5b}$$

These are the equations† of Dunn *et al.*[3] for the single-injection procedure. In the notation used by Wrenshall *et al.*,[4] R_A stood for R_1, R_D for R_2, N for $B_{(t)}$, and γ for i_t. EQUATIONS 4 and 5, in which F is zero, are seen to be identical with the differential equations used by these authors in evaluating from a C^{14}-glucose single-injection experiment, glucose inflow and outflow (before and after insulin) in a "central compartment" in which injected glucose mixes rapidly relative to its rate of inflow and outflow, namely:

$$R_A = -\frac{N}{\gamma}\frac{d\gamma}{dt} \tag{4c}$$

$$R_D = R_A - \frac{dN}{dt} \tag{5c}$$

When F is not zero, as in the priming dose-constant infusion experiments, integration of EQUATIONS 4 and 5 gives the expressions originally employed in our own work[5] except for the differences in the symbols employed, that is, for EQUATION 4:

$$\frac{i_t - \dfrac{F}{R_1}}{i_o - \dfrac{F}{R_1}} = \left(\frac{B_o}{B_t}\right)^{\frac{R_1 t}{B_t - B_o}} \tag{4d}$$

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* These equations and the preceding simple derivation were given to us by Bernard Altshuler of the Institute of Industrial Medicine at the New York University Medical Center, New York, N. Y. The equations replace the integrated form of the relationship that we had previously used,[5] giving exactly the same numerical values with less arithmetical effort.

† In EQUATION 3 of Dunn *et al.*,[3] which corresponds with EQUATION 5b of the present discussion, the letter t as denominator under $B_o - B_t$ was omitted in printing.

or when R_1 is zero (endogenous inflow stops):

$$i_t = i_o + \frac{Ft}{B_t - B_o} \ln \frac{B_t}{B_o} \tag{4e}$$

and for EQUATION 5:

$$R_2 = R_1 + \frac{B_o - B_t}{t} \tag{5}$$

EQUATION (4*e*) is used as an identity that must be satisfied if R_1 is to be zero, whereas if R_1 is not zero equation (4*d*) gives its value.

It is seen from the above that in those experiments with C^{14} glucose in which calculations have been made of glucose production (R_1) and utilization (R_2) after insulin injection that the same basic model and assumptions have been adopted by all investigators. However, all of them have not used the same method for determining the size of the glucose pool to be used in calculation.

Dunn *et al.*[3] have adopted the usual tacit assumption that intravenously injected glucose mixes rapidly with the whole of the glucose pool. On this basis they calculate pool size by extrapolating the curve of diminishing blood glucose-specific activity back to the time at which a single injection of C^{14} glucose of known total activity was given intravenously. Subsequent to the injection of insulin they use the whole of this pool in their calculations. Wrenshall *et al.*[4] do the same thing, although they speak of using a "central compartment" in which injected glucose mixes rapidly relative to its rate of inflow and outflow. In our own work[6] we have calculated the magnitude of the error in evaluating pool size that is incurred by extrapolating back to zero time the specific activity values observed between 60 and 180 min. on the assumption that these observed values refer to a well-mixed pool. We have demonstrated that this error goes through a minimum value when a continuous infusion of C^{14} glucose given at a certain rate follows the initial priming dose. In calculating events after insulin injection we have used[5] one half of the pool size as determined in this way by the "priming dose-constant infusion" technique because only about one half of the pool responds rapidly to changes in the rate at which C^{12} glucose enters from the liver.

The Influence of Injected Insulin

It is readily apparent from panels *A* and *B* of FIGURE 2 that the sensitivities of the "single-injection" (panel *A*) and the "priming dose-constant infusion" (panel *B*) methods in detecting changes in the rate of C^{12}-glucose production are similar. The same proportionate difference in observed specific activity is predicted for either technique when a reduction occurs in the rate of C^{12}-glucose output by the liver. It should be noted that in calculating the expected curves for panel *A* (single-injection experiment) no correction was made for the probable circumstance that the slowly mixing moiety of the glucose pool has a specific activity about 30 per cent higher than the rapidly mixing portion from which samples for observation are taken (compartments *Q* and *I* of Figure 4 of Steele *et al.*[6] represent the rapidly mixing half of the pool and *L* the slowly mixing half). In the event that there is no action of insulin to hasten the exit into cells of the glucose in the slowly mixing half of the pool, this higher specific-activity glucose should as time goes on mix with the fast half of the pool and raise the observed specific activity above the curves shown in panel *A*. It was felt to be more suitable for the present purpose of comparing the sensitivities of the two methods to accept the tenet under which the single injection calculations have been made, that is, instantaneous mixing throughout the glucose pool, whatever value may be taken for its size. However, it should

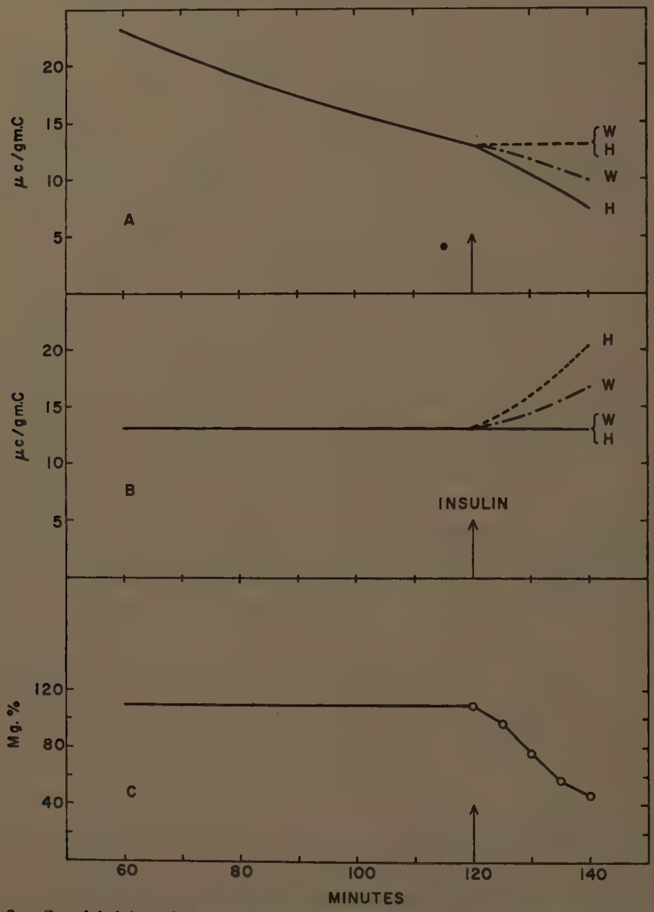

FIGURE 2. Sensitivities of single-injection (panel *A*) and priming dose-continuous infusion (panel *B*) methods for detection of diminution in hepatic C^{12}-glucose output after insulin injection. *Solid lines* denote continuation of C^{12}-glucose output at pre-insulin level; *broken lines* denote complete cessation of C^{12}-glucose output after insulin injection. *W* denotes that whole-pool, and *H* denotes that half-pool size was used in calculation. Panel *C* shows the plasma glucose concentrations on which the calculations were based; the insulin dosage was 0.1 unit/kg. body weight by intravenous injection. EQUATION 4 (*see text*) was used to calculate *A* and *B* for a typical dog of 24-kg. body weight with a whole-pool size prior to insulin of 2.45 gm. of glucose carbon and a turnover prior to insulin of 0.0233 gm. of glucose carbon per minute. For single-injection, 100 μc. C^{14} glucose was assumed given at zero time; for priming dose-constant infusion, 31.9 μc. C^{14} glucose was assumed given at zero time, followed by 0.303 μc. C^{14}/min. continuously.

be noted that the magnitude of the change that would be brought about by the entry of higher specific-activity glucose from the slow half of the pool is such as to bring the line *H* of FIGURE 2A up to coincide with the line *W* and to bring about an equal rise in the line *WH*. Under these conditions a leveling off of specific activity such as is seen in the experiments of Dunn *et al.*[3] would denote not a cessation but a diminution of C^{12}-glucose output from the liver; an actual upward movement of specific activity would be required if the cessation of C^{12}-glucose output were to be concluded to have occurred.* Inasmuch as the foregoing assumes a selective action of insulin on the fast half of the pool, for which there is no experimental evidence, these thoughts were not included in the construction of FIGURE 2A.

The finding of Dunn *et al.*[3] was that an unchanging specific activity occurs during the whole period of blood sugar reduction after a single dose of insulin given intravenously in a C^{14}-glucose "single-injection" experiment (FIGURE 2A). Because of this finding and in view of the fact that in their calculations instantaneous mixing is assumed to occur in the whole glucose pool, Dunn and her associates interpreted this finding to mean a complete cessation of glucose output. Under certain conditions as outlined above, this assumption would be an overestimate of the amount of diminution of glucose output. Wall *et al.*, found slight changes in specific activity, indicating a slight diminution of glucose output by the liver after insulin.[5] If these changes were to be calculated on the basis of whole-pool rather than half-pool size, they would represent a somewhat larger influence of insulin on hepatic glucose output than was reported.

However, this difference in emphasis brought about by the methods of calculation does not represent the entire difference between the results by the two methods of using C^{14} glucose. In this connection it should be noted that the single-injection procedure, under somewhat different physiological conditions, does not always give the above result. Thus, Tarding and Schambye[7] and we,† using anesthetized normal dogs, have not obtained the long plateau in specific activity lasting throughout the whole period of falling blood sugar level after insulin that was reported by Dunn *et al.*[3] for unanesthetized normal dogs. Wrenshall *et al.*,[4] using diabetic dogs, found only a small apparent effect of injected insulin on hepatic glucose output. Berson and his collaborators, using unanesthetized normal rabbits, at first reported[8] findings indicating cessation of glucose release after intravenous insulin, but later, reporting on a longer series of animals, found[9] the effect "usually short-lived and occasionally absent."

The differing findings in experiments that seem to be quite similar except for the way in which C^{14} glucose was administered are perhaps to be understood in the light of experiments reported recently by Shoemaker *et al.*[10] These workers used the single-injection procedure, and measured in the unanesthetized animal the changes in specific activity after insulin in the portal vein blood as well as in the hepatic vein blood. During the period of falling blood glucose concentration they observed a gentler fall in hepatic vein blood glucose-specific activity (the "plateau" effect), but also found that during this period the specific activity of the portal vein blood showed an even more marked deviation from its preinsulin course. Since in their experiment there was no infusion of C^{14} glucose after the first single injection, such an effect on portal glucose-specific activity indicated that glucose sequestered during an earlier part of the experiment when the circulating glucose had a higher specific activity was re-entering the blood after insulin injection. The explanation advanced by Shoemaker *et al.* was that insulin facilitates re-entry into the blood of glucose sequestered by the cells of the intestinal mucosa. For the model experiment shown in FIGURE 2A it can be calculated* that the amount of sequestered glucose required to be returned to the circulating blood to give the 20-min. plateau after insulin on the assumption that hepatic C^{12}-glucose output continues undiminished is at least 9 per cent of the glucose utilized during the 120-min. period of the experiment prior to insulin injection, provided this sequestered glucose has the mean specific activity of the plasma glucose prior to insulin. This is a large amount of glucose, but perhaps not outside the realm of the possible.

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* This possibility was expressed to me by G. L. Searle and M. S. Goldstein in the spring of 1958.

† Unpublished experiments performed in collaboration with J. S. Bishop and R. Levine.

In very recent experiments of our group that are presented by de Bodo *et al.* elsewhere in this monograph[11] it was found that in some but not all cases a continuous slow intravenous infusion of insulin during a "priming dose-constant infusion experiment" brings about a considerable effect on specific activity in the direction expected as a result of diminution of C^{12}-glucose output by the liver. It was under similar circumstances (subcutaneously injected insulin) that the group associated with Weinhouse also obtained an accentuated apparent action of insulin on hepatic glucose output[12, 13] that in their experiments seemed to be sufficient to account for the whole of the blood sugar-reducing action, leaving essentially no residue for peripheral action of insulin. Thus, our own results using the priming dose-continuous infusion technique are in this respect similar to those obtained with the single-injection techniques, but consistently indicate a lesser apparent effect of insulin on hepatic glucose output.

Taking into consideration the sequestering effect noted by Shoemaker *et al.*,[10] it seems possible that the difference in results by the two techniques is due to the fact that in the single-injection procedure the sequestered glucose has a higher specific activity relative to the specific activity prevailing in the circulating glucose at the time insulin is administered. In the priming dose-continuous infusion procedure there is also a period after the priming dose is injected when the circulating glucose has a high specific activity relative to the final level determined by the continuous C^{14}-glucose infusion but, with this technique, the difference between the initial and the 2-hour specific activities is only about one third as much as in the single-injection procedure (see FIGURE 2 and its legend, which gives the amounts of C^{14} injected initially in the two cases).

In summary, the factors contributing to the changes in specific activity after insulin injection that seem to indicate an immediate diminution of hepatic glucose output are not yet fully understood. Further elucidation of these factors is required before the C^{14}-glucose technique can be stated to provide positive evidence for an action of insulin to inhibit hepatic glucose output.

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* The making of this calculation was suggested by conversations with Arnold Dunn.

The Influence of Endogenous Insulin Evoked by Injection of Glucose

It has been mentioned above that in calculation from the plasma glucose-specific activities and concentrations after insulin we have adopted the use of one half of that glucose pool size indicated by extrapolation of the 60- to 180-min. preinsulin-specific activities back to zero time. The reason for beginning observations 60 min. after the initial priming injection of C^{14} glucose is that prior to that time the complete mixing of the injected glucose with the whole of the body glucose pool has not been approached closely enough. This phenomenon has been submitted[6] to mathematical analysis, using a model of the body glucose pool consisting of a central plasma compartment and two pheripheral tissue fluid compartments.

On this basis it was concluded that one of the compartments, the glucose of which, together with the glucose of plasma, makes up about one half the total pool, mixes quite rapidly with plasma glucose, whereas the other peripheral compartment mixes slowly.

As a consequence of this picture of the glucose pool it was suspected that the intravenous injection of untagged glucose is followed by a plateau in specific activity,[14, 15] not because the liver stops producing C^{12} glucose, but because it continues to produce C^{12} glucose so as to balance the C^{14} glucose of higher specific activity entering during equilibration from the slower mixing part of the glucose pool. An obvious way to test this was to give a glucose load tagged with C^{14} to the same extent as the glucose already present in the body pool; when this was done, there was no evidence of cessation of C^{12}-glucose output by the liver subsequent to intravenous injection of a glucose load.[11, 16, 17] To complete the picture, a C^{12}-glucose load was administered to an eviscerated dog to which had been given a single injection of C^{14} glucose after its liver had been replaced by a constant-infusion pump delivering C^{12} glucose at a rate sufficient to maintain a steady plasma glucose concentration. A typical result, obtained in each of four such experiments carried out in collaboration with Jonathan S. Bishop and Rachmiel Levine,[18] is shown in FIGURE 3. It is clear that stoppage of the steady input of glucose is not necessary to get a plateau in such an experiment, and the pattern of specific activity obtained is consistent with the idea that there is a slowly mixing part of the body glucose pool as concluded earlier[6] from our experience with injected C^{14} glucose.

A practical advantage of the priming dose-continuous infusion technique in connection with glucose load experiments is the fact that injection of a glucose load tagged to the same extent as the glucose already circulating is facilitated. To our knowledge, this kind of experiment has not been done using the single-injection technique, but it is certainly not impossible that it could be accomplished. Consequently in FIGURE 4, in which the sensitivites of the two techniques are compared, the disturbing influence of the injection of a C^{12}-glucose load has been eliminated by assuming that in both the single injection (panel *A*) and the priming dose-continuous infusion (panel *B*) procedures the glucose load is given instantaneously at the specific activity prevailing in the plasma glucose at that moment. It is seen in FIGURE 4 that by either method a change in rate of C^{12}-glucose production by liver is more difficult to detect than in the insulin experiments because the glucose pool whose specific activity must be affected is larger, but again it is seen that there is no difference between the two methods as regards their sensitivity for detection of such a change. Furthermore, since the actual experiment using the priming dose-continuous infusion technique results in very little change in specific activity, the importance of

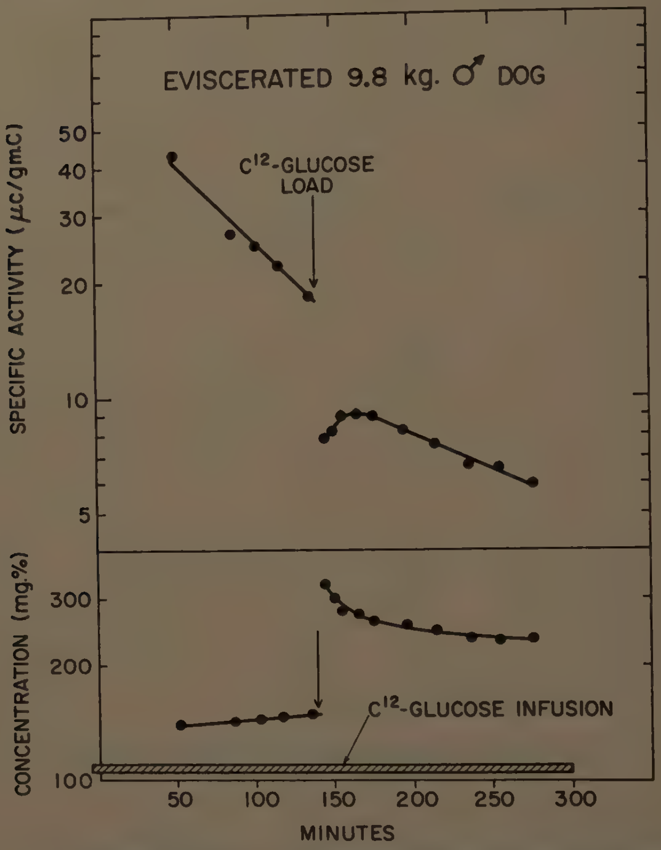

FIGURE 3. Experiment of Steele *et al.*,[18] showing changes in plasma glucose-specific activity after intravenous glucose in a single-injection experiment using C^{14} glucose in an eviscerated dog whose blood glucose level was sustained by constant infusion of C^{12}-glucose. At zero time 50 μc. of C^{14} glucose was given intravenously; at the time indicated by the arrow a glucose load (200 mg./kg. body weight) was given intravenously. The sustaining C^{12}-glucose constant infusion was at 120 mg./kg./hr. Reproduced by permission from *The American Journal of Physiology.*

whether the whole-pool size or the half-pool size is used in calculation is minimized in experiments using this technique.

With regard to the physiological significance of the finding that a glucose-load hyperglycemia does not cause a diminution of hepatic C^{12}-glucose output, it is clear that there is no longer any evidence to indicate an immediate effect of endogenously secreted insulin to decrease hepatic glucose output. However, there is some doubt about the converse conclusion, namely, that this experiment demonstrates that one should expect a lack of action of injected insulin on hepatic glucose output. The reason for this is that, although there is very likely some extra endogenous insulin secreted in such an experiment, the amount of extra insulin thus secreted is unknown within very wide limits; perhaps the amount is too little to bring about an otherwise demonstrable effect of purified insulin preparations.

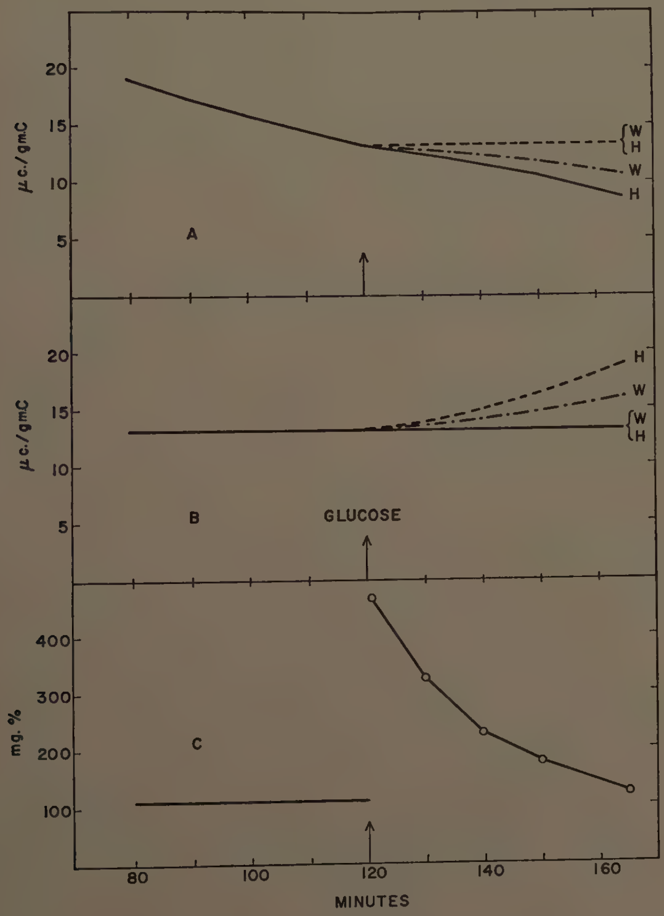

FIGURE 4. Sensitivities of single-injection (panel *A*) and priming-dose continuous-infusion (panel *B*) methods for detection of diminution in hepatic C^{12}-glucose output after intravenous injection of a tagged glucose load (*see text*). *Solid lines* represent the continuation of C^{12}-glucose output at the preload level; *broken lines* represent complete cessation of C^{12}-glucose output by liver after the glucose load. *W* denotes that whole-pool, and *H* denotes that half-pool size was used in calculation. Panel *C* shows the plasma glucose concentrations on which the calculations were based; these were adopted from an experiment in which 700 mg. glucose/kg. body weight was given intravenously over a 10-min. period. For other characteristics of the glucose pool used in calculations, see the caption to FIGURE 2.

Summary

Differences in the observed results as well as differences in interpretation of results of the various C^{14}-glucose methods seem to be responsible for the differing conclusions that have been reached regarding an immediate inhibition by insulin of hepatic glucose output. Artifacts may affect the results more when C^{14} glucose is administered in one way than when it is administered in another. It is probable that differences in the physiological states of the experimental animals are involved in other instances of varying results.

The weight of the evidence bears out the view that inhibition of hepatic glucose output is not the most important factor in the immediate blood sugar-lowering action of injected exogenous insulin. With regard to the question, important from the theoretical standpoint, of whether injected insulin exerts any immediate influence on hepatic glucose output, it must be concluded that further elucidation is required of the factors capable of influencing specific activity after exogenous insulin is injected before the C^{14}-glucose method can be relied upon to answer this question. As regards the extra endogenous insulin secretion evoked by glucose loading, hepatic inhibition is not revealed in this instance by the C^{14}-glucose experiments. Uncertainty as to the amount of extra insulin actually secreted renders this an unsatisfactory indication that one should expect a complete absence of any direct hepatic inhibitory effect of purified insulin preparations. However, this finding does seem to indicate that endogenous insulin secreted at a physiological rate via pancreatic venous blood does not act by direct inhibition of hepatic glucose output.

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EFFECTS OF EXOGENOUS AND ENDOGENOUS INSULIN ON GLUCOSE UTILIZATION AND PRODUCTION*

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Recent findings reported in the literature concerning the mechanism of insulin action are in some respects contradictory. The observations originally made by Gemmill[1-3] that the addition of insulin to the incubation medium enhances the glucose uptake and glycogen formation by the isolated diaphragm were amply confirmed by subsequent investigators.[4] From these and other observations it has been postulated that endogenous insulin likewise increases glucose utilization by peripheral tissues. More recently, Renold *et al.*,[5] studying the alterations of carbohydrate metabolism in diaphragm and in liver slices after the administration of insulin to diabetic rats for varying periods of time, found that insulin acted within minutes to increase glucose utilization (glucose uptake and glycogen formation), only in the peripheral tissues; the effects on the liver appeared only when the liver was extirpated many hours following injections of insulin into the diabetic animal. This led to the conclusion that the "immediate metabolic effects of insulin [are] at the periphery only."

On the other hand, it has been suggested in several recent studies that the major, if not the exclusive, action of insulin is to inhibit hepatic glucose release. This suggestion is based on the changes in plasma glucose production and utilization observed following a subcutaneous injection of insulin into humans who received a single trace dose of C^{14} glucose[6] and on arterial-hepatic-venous differences in blood glucose concentration during the course of insulin infusion (L. L. Madison, B. Combes, W. Strickland, R. Unger, and R. Adams, Unpublished data). It was postulated further that endogenous insulin secreted under physiological conditions exerts a similar effect on hepatic glucose release.[6]

The studies reported here represent a continuation of earlier work[7] undertaken to determine whether insulin exerts its action on the peripheral tissues to bring about increased removal and utilization of plasma glucose or on the liver to diminish the glucose production and release to the plasma, or both. The experiments were carried out on dogs without the use of general anesthesia and without any operative procedure, under truly physiological conditions, using C^{14} glucose.

PRELIMINARY CONSIDERATIONS

Employing minute amounts of radioactive glucose, the size and turnover rate of the body glucose pool were measured in unanesthetized animals in the postabsorptive steady state.[8,9] The size of the body glucose pool is defined

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* The investigations reported in this paper and carried out at the New York University College of Medicine were supported by Research Grant A-113(C7) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., by a grant-in-aid from the American Cancer Society, Inc., New York, N. Y., and by a research grant from Eli Lilly and Company, Inc., Indianapolis, Ind. Part of the research was conducted at the Brookhaven National Laboratory, Upton, N. Y., under the auspices of the Atomic Energy Commission, Washington, D. C.

as the amount of glucose that dilutes the administered C^{14} glucose; the turnover rate of the glucose pool as the rate of removal of plasma glucose by the tissues (glucose utilization) and the rate of replacement of plasma glucose (glucose production by the liver). In the resting postabsorptive state, when the plasma-glucose concentration remains constant and no glucose is excreted in the urine, glucose utilization and production are equal.

In the experiments reported here, the body glucose pool was labeled by an intravenous priming injection of a trace amount of uniformly labeled C^{14} glucose. The established radioactivity of the pool was then maintained by a constant intravenous infusion of a trace amount of C^{14} glucose that balanced the C^{12} glucose released by the liver as long as this did not change (FIGURE 1, *upper left*). From the course pursued by the plasma-glucose specific activity the size and turnover rate of the body glucose pool were determined.

In a number of experiments insulin was injected during the course of constant infusion of C^{14} glucose.[7] If the resulting decrease in the size of the body glucose pool and plasma-glucose concentration were due to an increased glucose utilization by the tissues without affecting the release of glucose from the liver, the specific activity would remain unchanged during the fall of the plasma-glucose concentration (FIGURE 1, *upper right*). Since in this case the glucose inflow from the liver does not change, the C^{14} glucose infused by constant infusion still balances the endogenous C^{12} glucose released by the liver. If, however, the fall in plasma-glucose concentration were due to a decrease in glucose production, then the infused C^{14} glucose would no longer be balanced by the endogenous C^{12} glucose inflow from the liver, and the specific activity of the plasma glucose would rise during the period of development of the hypoglycemia (FIGURE 1, *lower left*). A fall in specific activity indicates that there is an increased C^{12} glucose inflow from the liver (FIGURE 1, *lower right*). This is shown very markedly when the plasma-glucose concentration returns from the insulin-induced hypoglycemic level to its preinsulin level, signifying that this process is due to an increased glucose release from the liver.

The method just described, consisting of priming injection followed by constant infusion of a trace amount of C^{14} glucose, gives more accurate results for glucose pool size and turnover rate in the preinsulin period than either the single-injection technique or a technique involving continuous infusion without a priming dose. In a critical analysis[8] the magnitude of the errors incurred in using these various procedures of administering the C^{14} glucose was evaluated. During the period following the administration of either insulin or a glucose load, the only circumstance under which perfectly accurate calculated glucose inflows from the liver can be expected is in the event that, first, the whole glucose pool is initially homogeneous in specific activity, and second, that the plasma-glucose specific activity does not change with time. In the period of falling blood sugar level, these requirements are better satisfied, by the continuous-infusion techniques than by the single-injection procedure. When recovery from hypoglycemia (in the case of the insulin experiments) takes place and the plasma-glucose specific activity does fall markedly as a result of increased hepatic glucose output, our practice of using the half-pool rather than the whole-pool size in making calculations of glucose inflow minimizes the

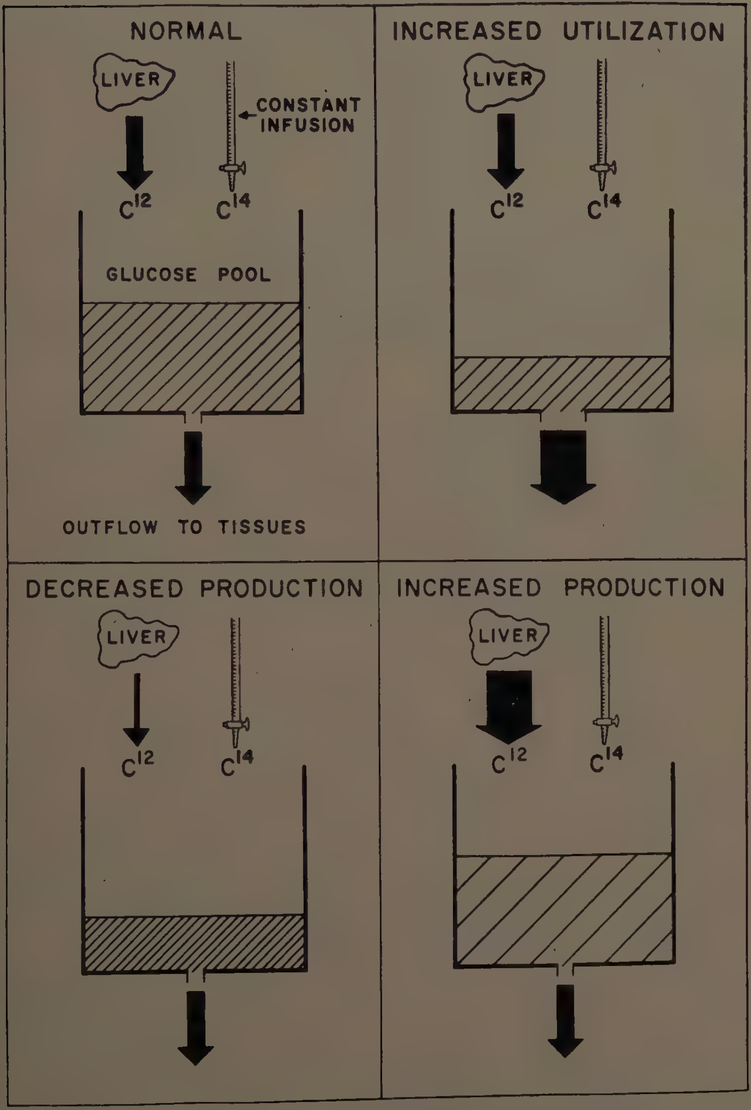

FIGURE 1. Diagram showing the relationship of changes in size of body glucose pool and plasma-glucose specific activity to changes in glucose production and utilization. The density of diagonal lines is proportional to specific activity. *Upper left*: normal postabsorptive state. The specific activity of the glucose pool is established by a priming injection, and is maintained by matching C^{12} glucose inflow from liver with constant infuson of C^{14} glucose. Glucose production equals glucose utilization. *Upper right*: changes produced if insulin injection lowered glucose pool by increasing only glucose outflow to tissues; the pool size decreases, but specific activity is unaltered. *Lower left*: changes if insulin acted only to decrease glucose inflow from liver; the pool size decreases, but specific activity increases, since the infusion of C^{14} glucose is continued. *Lower right*: increased inflow of hepatic glucose into the body glucose pool previously diminished by insulin; pool is elevated, and specific activity is diminished since increased hepatic glucose inflow is not matched by constant C^{14} glucose infusion.

errors caused by the fact that new glucose introduced into the blood mixes rapidly with only about one half of the total glucose pool.[7] With regard to recycling, by which is meant the reincorporation into glucose of fragments containing tagged glucose carbon removed earlier from the blood by the tissues, the continuous-infusion methods minimize the disturbing effect of this phenomenon, inasmuch as the returning tagged fragments are entering a pool maintained at a higher level of specific activity than in the single-injection procedure. The same consideration applies to glucose, which might be sequestered earlier in the experiment as intracellular glucose (in some specialized cells) or as glycogen; the reintroduction of such tagged molecules later in the experiment will have a lesser disturbing effect on calculated inflow in continuous-infusion experiments than in single-injection experiments.

MATERIALS AND METHODS

The experiments were performed on trained adult normal and hypophysectomized dogs in the postabsorptive state (17 to 18 hours fasted) without the use of anesthesia. The dogs were maintained on a standard diet.[10]

The body glucose pool was labeled by a "priming" injection of uniformly-labeled C^{14} glucose into the saphenous vein. The established plasma-glucose specific activity was then maintained for several hours by a constant infusion into the external jugular vein of the same C^{14} glucose at a rate that matched the inflow rate of C^{12} glucose from the liver into the plasma, provided the inflow remained unchanged.[8] The C^{14}-glucose infusion was also continued uninterrupted during infusion of insulin or glucose loads.

In the experiment wherein insulin was administered, the procedure was as follows. After the C^{14} glucose had been administered for 2 hours and control values had been obtained, trypsin-treated crystalline zinc insulin (Lilly; low in glucagon content*), freshly dissolved in saline at *p*H 3, was administered intravenously either by a single injection or by a prolonged infusion for periods of 90 to 135 min. The dosages of insulin and rates of infusion were varied and are indicated in the various figures. The constant infusion of the trace amounts of C^{14} glucose continued throughout the entire experiment.

In the experiments wherein secretion of endogenous insulin was evoked, a glucose load was administered at 2 hours during the course of the C^{14}-glucose infusion. The glucose load was of the same specific activity as the plasma glucose. The dosage was either 0.75 gm./kg., infused as a 30 per cent solution in 10 min., or 0.166 gm./kg. as a 10.7 per cent solution infused in 60 min.

At various intervals samples of blood were drawn from an exposed femoral vein, delivered into heparinized tubes and centrifuged, and the plasma immediately separated. The plasma-glucose concentration of each sample was determined, using aliquots of Somogyi zinc-barium filtrate[11] by two methods, those of Hagedorn and Jensen[12] and Nelson.[13] A sample of glucose from each filtrate was isolated as the phenylosazone derivative and the glucose specific activity determined by either of two methods. In earlier experiments, the derivative was burned to CO_2 by wet combustion, and the $C^{14}O_2$ counted by the method of Van Slyke *et al.*[14] In later experiments, the phenylosazone derivative was converted to the glucosotriazole derivative, and the glucose-specific activity was determined by the liquid scintillation counting method of Steele *et al.*[15] The method for calculation of the glucose pool size and the rates of plasma glucose production and utilization in the steady state[8] and during periods of changing plasma-glucose concentration[7] are given elsewhere.

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* We are indebted to O. K. Behrens, Eli Lilly and Company, Indianapolis, Ind. for his donation of the insulin.

RESULTS

Effect of Insulin Administered by a Single Injection into a Peripheral Vein of the Normal Dog

After a 2-hour preliminary period, during which C^{14} glucose was administered as described above to determine the control size and turnover rate of the glucose pool, insulin (low in glucagon content) was administered as a single injection into either the saphenous or femoral vein. FIGURE 2 shows the data obtained in a typical experiment. The dosage of insulin in this instance was 0.063 U./kg. intravenously, but similar results were obtained in other experiments with the dosage varying between 0.025 U./kg. and 0.25 U./kg. The observed plasma-glucose concentrations and plasma-glucose specific activity values are recorded. The calculated rates of glucose inflow into the plasma (glucose production) and glucose outflow from the plasma (glucose utilization) are also shown. It can be seen that the decrease of plasma glucose concentration subsequent to the injection of insulin is accompanied by a slight elevation of the plasma-glucose specific activity. The calculated rate of glucose uptake by the tissues shows a marked increase, while the calculated rate of glucose production by the liver shows a small transient decrease. Thus, it appears that the insulin-induced hypoglycemia is due mainly to an increased glucose utilization and only to a lesser degree to a decreased glucose production.

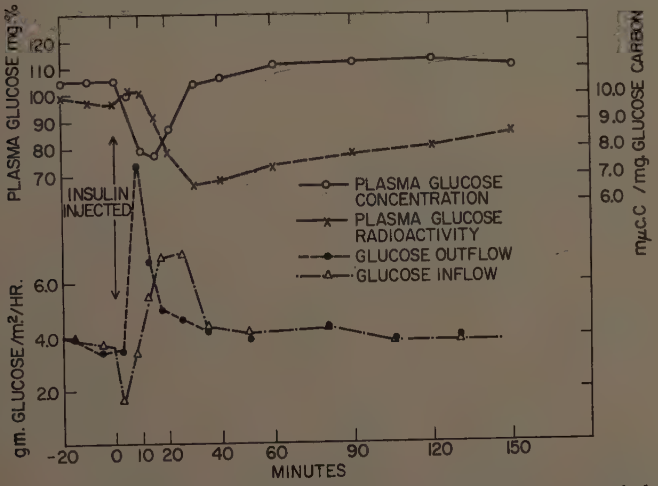

FIGURE 2. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in a normal dog following intravenous injection (0.063 U./kg.) of trypsin-treated insulin (Lilly). Priming dose of C^{14} glucose injected 120 min prior to zero time. A trace amount of C^{14} glucose was infused intravenously during the entire experiment. Reproduced by permission from the *American Journal of Physiology*.[7]

It can also be seen in FIGURE 2 that during the recovery period the return of the plasma glucose concentration to its preinsulin level is accompanied by a considerable decrease in the plasma-glucose specific activity. The glucose production by the liver is greatly increased during this period and, since the glucose utilization does not decrease below the preinsulin level, the return of the plasma-glucose concentration to control level is due entirely to the increased glucose production.

With larger doses of insulin the resulting hypoglycemia was more marked, and the glucose utilization was increased to a greater extent and for a longer period than following smaller doses of insulin. The rate of enhancement of glucose production by the liver following insulin injection was related to the degree of hypoglycemia. However, with the larger doses of insulin the plasma-glucose concentration decreased to lower levels than with smaller doses before the increased glucose release by the liver was evoked. Thus, there did not appear to be a critical level of hypoglycemia at which the liver started to release increased amounts of glucose.

Effect of Insulin Administered by a Prolonged Constant Infusion into a Peripheral Vein of the Normal Dog

In view of the finding that the depancreatized dog requires a constant infusion of insulin in order to maintain a normal blood sugar level,[16-19] it may be assumed that insulin is continually being secreted in the normal animal even in the postabsorptive state.[20] Thus it was thought that insulin administered by continuous infusion instead of single injection would produce effects that would more closely resemble those occurring during the physiological secretion of insulin. In a series of experiments carried out on normal dogs after a preliminary 2-hour control period, insulin was infused for a prolonged period at a constant rate into a peripheral vein, the external jugular vein.

FIGURE 3 shows the results of an experiment in which 0.1 U./kg. of insulin was infused during 90 min. It can be seen that the plasma-glucose concentration fell during the insulin infusion and that the hypoglycemia persisted throughout the period of insulin infusion. The corresponding plasma-glucose specific activity remained remarkably uniform at the preinsulin level. The rate of glucose uptake by the tissues was greatly increased during the insulin infusion and appeared to be entirely responsible for the fall in plasma glucose, inasmuch as the glucose production by the liver remained approximately at the preinsulin level. The latter finding is the more noteworthy since even the severe hypoglycemia did not evoke a significant increase in glucose production by the liver.

Upon the termination of the insulin infusion, the plasma-glucose concentration rose rapidly and returned to its preinsulin level. This change was accompanied by a significant drop in the plasma-glucose specific activity. The rate of glucose production by the liver increased markedly and was responsible for the return of the plasma-glucose concentration to the preinsulin value since the glucose utilization did not decrease below its control value and indeed was still elevated during this period.

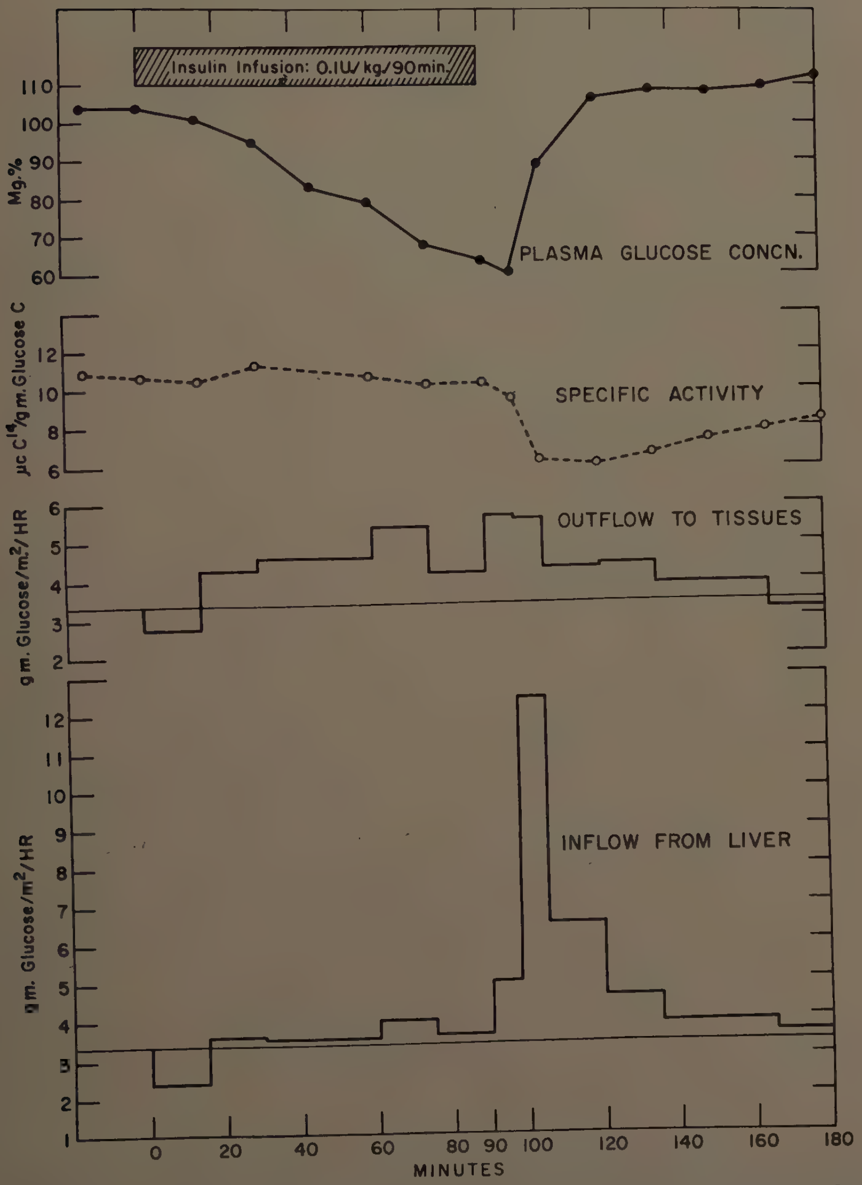

FIGURE 3. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver (in grams of glucose per square meter surface area per hour[7-9]) in a normal dog given an intravenous infusion of insulin, 0.1 U./kg. in 90 min. The light reference lines across graph are preinsulin inflow and outflow rates. C^{14} glucose was administered as in FIGURE 2. C^{14} priming dose 30 μc.; infusion 0.274 μc./min. It can be seen that during insulin infusion the decrease in the plasma glucose concentration was due mainly to an increased outflow of plasma glucose to the tissues. The inflow of glucose from the liver into the plasma did not increase, despite the presence of hypoglycemia, until after termination of the insulin infusion. Reproduced by permission from *Metabolism*.[28]

FIGURES 4 and 5 show the results of 2 experiments in both of which 0.25 U./kg. of insulin was infused intravenously in 90 min. As expected, in both cases the plasma-glucose concentrations fell more rapidly and to lower levels than in FIGURE 3, where a smaller dose of insulin (0.1 U./kg.) was used. The plasma-glucose concentrations remained at the low levels during the insulin infusions. In both expriments during the development of hypoglycemia there was a transient rise in the plasma-glucose specific activity. The rates of glucose utilization were markedly increased. The rates of glucose production shortly after the start of insulin infusion decreased, to a greater extent in the experiment shown in FIGURE 5 than in that shown in FIGURE 4, indicating that, at least in these experiments, the initial fall in plasma-glucose concentration was due to both an increased glucose utilization and diminished glucose production.

During the course of the insulin infusion, when the plasma-glucose concentrations remained at the low levels, the glucose utilization continued at increased rates. At the same time the rates of glucose production were also increased, but not adequately enough to elevate the plasma-glucose concentrations.

As in FIGURE 3, in these experiments the termination of insulin infusion was followed by a rapid rise of the plasma-glucose concentration to control levels, and this was accompanied by a considerable reduction of the plasma-glucose specific activity values. The marked rise in glucose production in FIGURES 4 and 5 was responsible for the return of the plasma-glucose concentrations to the preinsulin levels. The rates of glucose utilization were still elevated during this period in both experiments.

The experiment shown in FIGURE 6 differed from those shown in FIGURES 4 and 5 in several respects. First, the rate on insulin infusion during the initial 20 min. was double (0.0061 U./kg./min.) the rate of infusion in the experiments in FIGURES 4 and 5 (0.0028 U./kg./min.), resulting in a more precipitous fall of plasma-glucose concentration. This was again accompanied by a transient rise in specific activity. It appears that the development of the hypoglycemia was again due to an increased glucose utilization and to some extent to a decreased glucose production by the liver.

After the initial 20 min. the rate of insulin infusion was reduced to 0.0028 U./kg./min., which was the same as the rate of infusion shown in FIGURES 4 and 5. As shown in FIGURES 4 and 5, this rate of insulin infusion was maintained for 90 min., whereas in FIGURE 6 it was given for 115 min. in addition to the higher rate during the initial 20 min.

The marked increase in glucose production appeared as in previous experiments only after the insulin infusion had been terminated (135 min.) and again was responsible for the return of the plasma-glucose concentration to control levels.

In one dog, insulin was injected subcutaneously (0.15 U./kg.). The hypoglycemia developed slowly, reached the lowest concentration at 80 min. and continued at this level until the termination of the experiment at 120 min. The attending changes in glucose utilization and production were similar to those observed when insulin was administered by intravenous infusion. Thus,

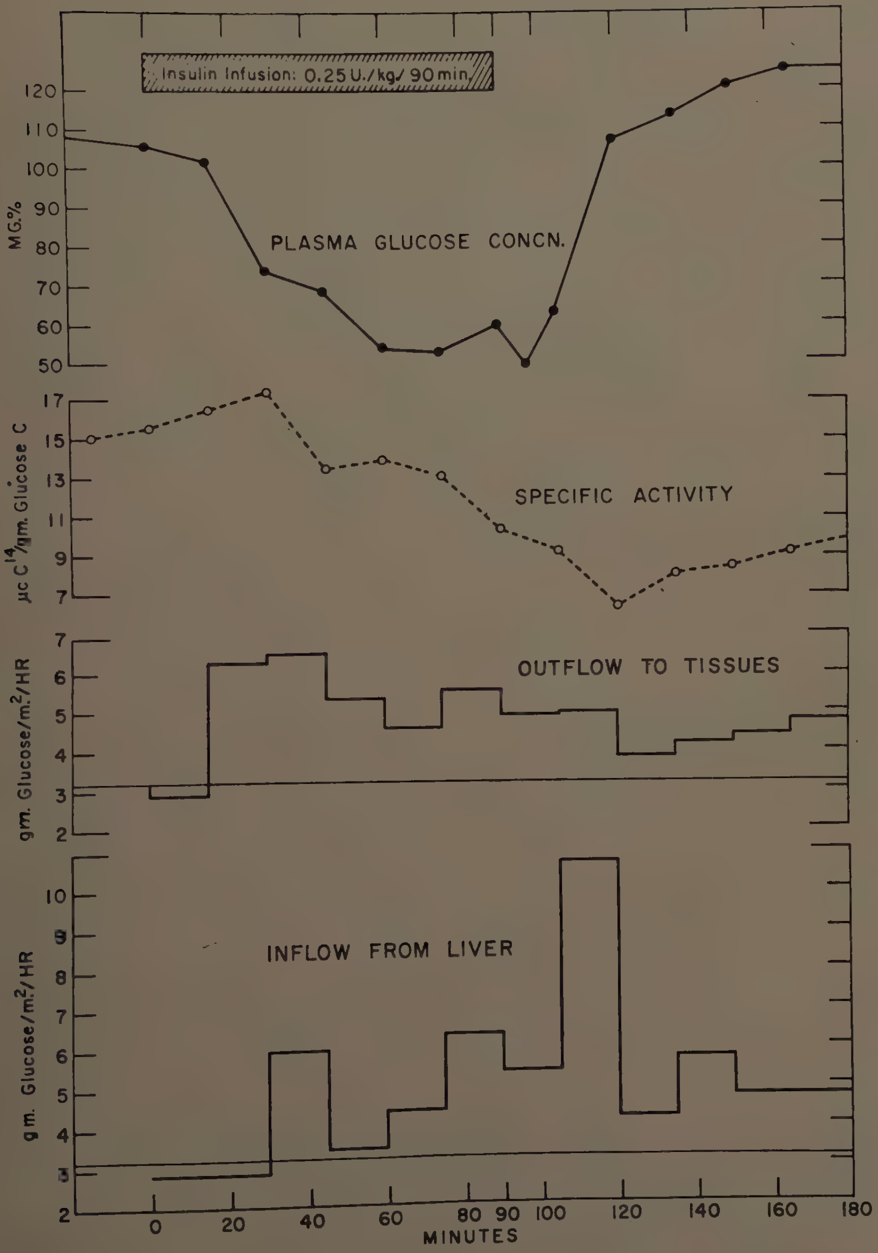

FIGURE 4. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in a normal dog given an intravenous infusion of insulin (0.25 U./kg. in 90 min.). The lighter reference lines across graph are preinsulin inflow and outflow rates. C^{14} glucose was administered as in FIGURE 2. C^{14} priming injection 30 μc.; infusion 0.278 μc./min. The hypoglycemia was due amost entirely to an increase in rate of glucose outflow to tissues. The glucose inflow from the liver during the insulin infusion was increased from time to time but not sufficiently to elevate the plasma-glucose concentration. The glucose inflow increased significantly shortly after termination of the insulin infusion and the plasma-glucose concentration was rapidly elevated. Reproduced by permission from *Metabolism.*[28]

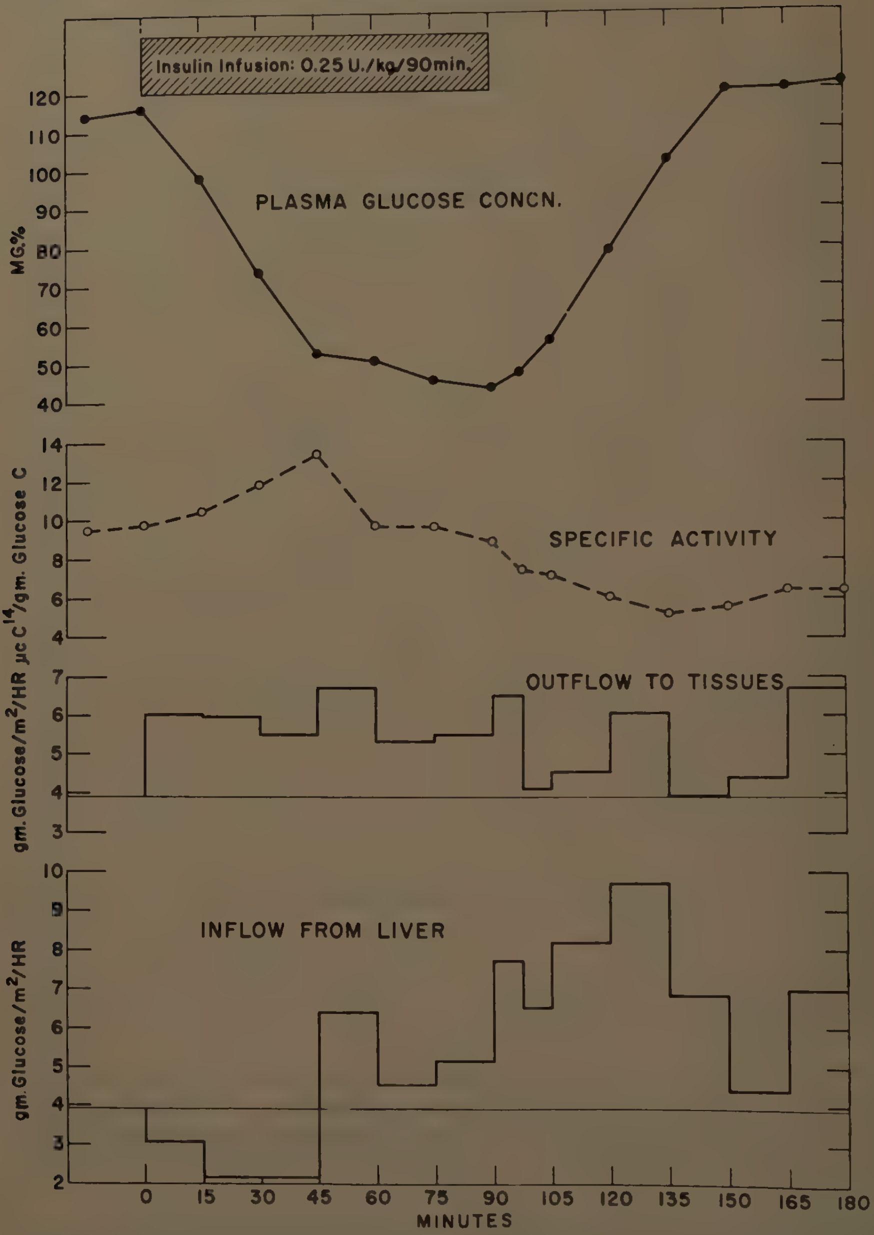

FIGURE 5. Plasma-glucose concentrations and specific activities, rates of glucose outflow and inflow from liver in a normal dog given an intravenous infusion of insulin (0.025 U./kg. in 90 min.). Other information as in legend for FIGURE 3. C^{14} priming dose 30 μc.; infusion 0.283 μc./min. The glucose inflow shortly after start of insulin infusion decreased to greater extent than in FIGURE 4.

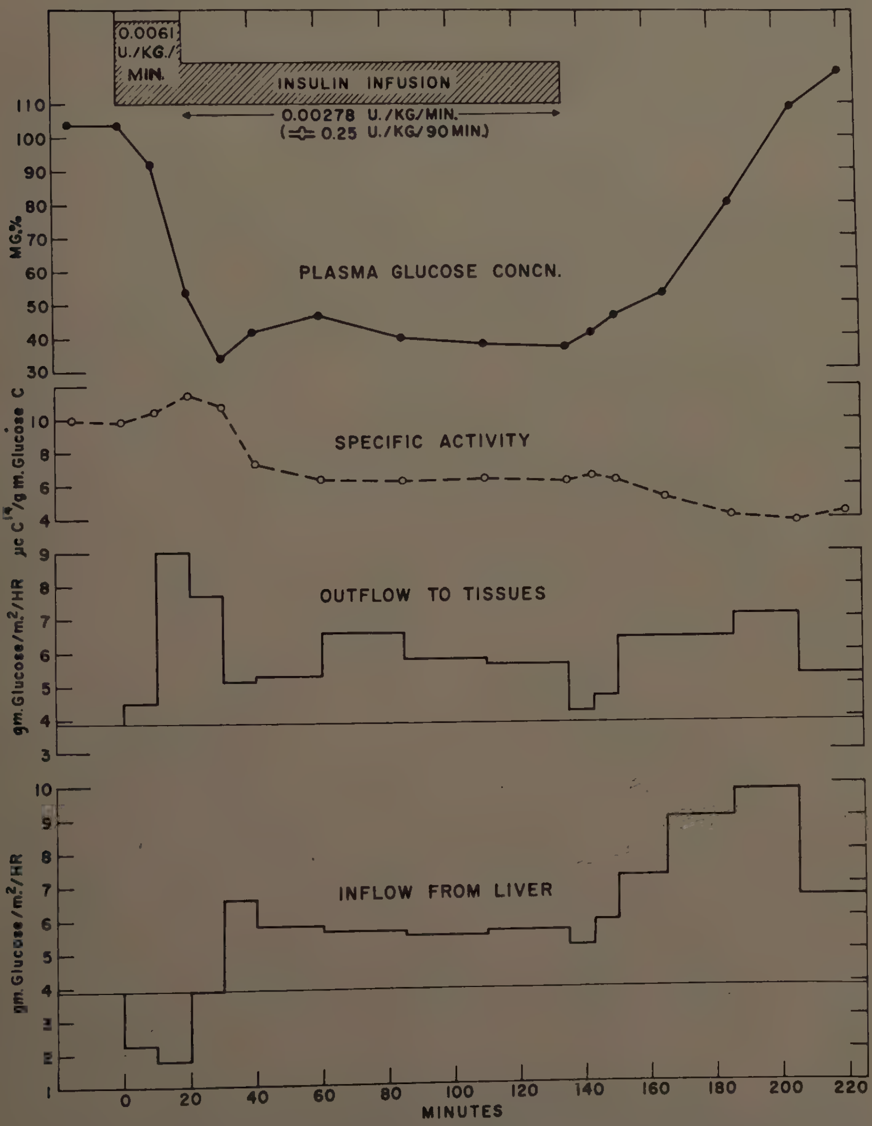

FIGURE 6. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in a normal dog given an intravenous infusion of insulin: initially at a faster rate, 0.0061 U./kg./min. for 20 min., and then at the same rate as in FIGURES 4 and 5, 0.0028 U./kg./min., but for 115 min. Other information as in legend for FIGURE 3. C^{14} priming dose, 30 μc.; infusion, 0.222 μc./min. The plasma-glucose concentration fell precipitously with the larger dose of insulin and stabilized at hypoglycemic levels with the subsequent reduced dosage of insulin. The specific activity rose transiently at the beginning. The fall in glucose level was due to increased glucose outflow and, to some extent, to diminished glucose inflow. The glucose inflow from liver increased during the insulin infusion, but was markedly increased following cessation of insulin infusion and elevated the plasma-glucose levels.

the development of hypoglycemia was due mainly to an increase in glucose utilization. The glucose production fluctuated during this period, but significantly it did not increase above normal during the period of sustained hypoglycemia, indicating a restraining effect of insulin.

Effect of a Glucose Load Administered into a Peripheral Vein of the Normal Dog

Endogenous insulin released under physiological conditions traverses the liver before reaching the peripheral circulation. In view of this, it was desirable to compare the effects on glucose production and utilization observed during exogenous insulin infusion with those obtained as a result of increased secretion of endogenous insulin. The secretion of endogenous insulin was evoked by intravenous infusion of a glucose load. Using the same procedure as in preceding experiments, the body glucose pool was labeled with a priming injection of trace amounts of C^{14} glucose, and the specific activity was maintained by a constant infusion of C^{14} glucose. After a 2-hour period that served to establish control values for the size and turnover rate of the body glucose pool, a glucose load of the same specific activity as that of the circulating plasma glucose was infused intravenously. The infusion of the trace amounts of C^{14} glucose continued without interruption and, therefore, alterations in glucose specific activity would reflect changes in hepatic glucose release as discussed above under PRELIMINARY CONSIDERATIONS.

In the experiment shown in FIGURE 7, the glucose load labeled with C^{14} glucose (0.75 gm./kg.) was infused into the saphenous vein in 10 min. and resulted in a hyperglycemia that lasted for about 50 min. During the hyperglycemia the specific activity of the plasma glucose showed a tendency to rise slightly. As expected, the glucose load resulted in a great enhancement of utilization of plasma glucose. The inflow of glucose from the liver was but slightly reduced during the period of hyperglycemia. It is readily apparent from FIGURE 7 that the return of the plasma-glucose concentration from the hyperglycemia to control levels was accomplished entirely by an increased removal of the glucose from the plasma. Some of this glucose was excreted in the urine, but this accounted for only 8 per cent of the glucose load.

In other experiments glycosuria was avoided by administering the glucose load over a prolonged period. In the experiment shown in FIGURE 8, the glucose load, again of the same specific activity as the plasma glucose, was administered by intravenous infusion at a dosage of 166 mg./kg. in 60 min. A moderate hyperglycemia was evident throughout the experiment. The plasma-glucose specific activity values showed some fluctuations that reflect changes in glucose release from the liver. The infusion of the glucose load resulted in an increased rate of glucose removal from the plasma. The glucose inflow from the liver was only slightly affected by the hyperglycemia. It is evident that the decrease in the plasma-glucose concentration from the hyperglycemic level was due entirely to increased glucose utilization by the tissues.

Since it is generally accepted that hyperglycemia evokes the secretion of insulin, one may conclude from the experiments in FIGURES 7 and 8 that the effects of physiologically released insulin on glucose utilization and production are similar to the effects produced by administration of insulin.

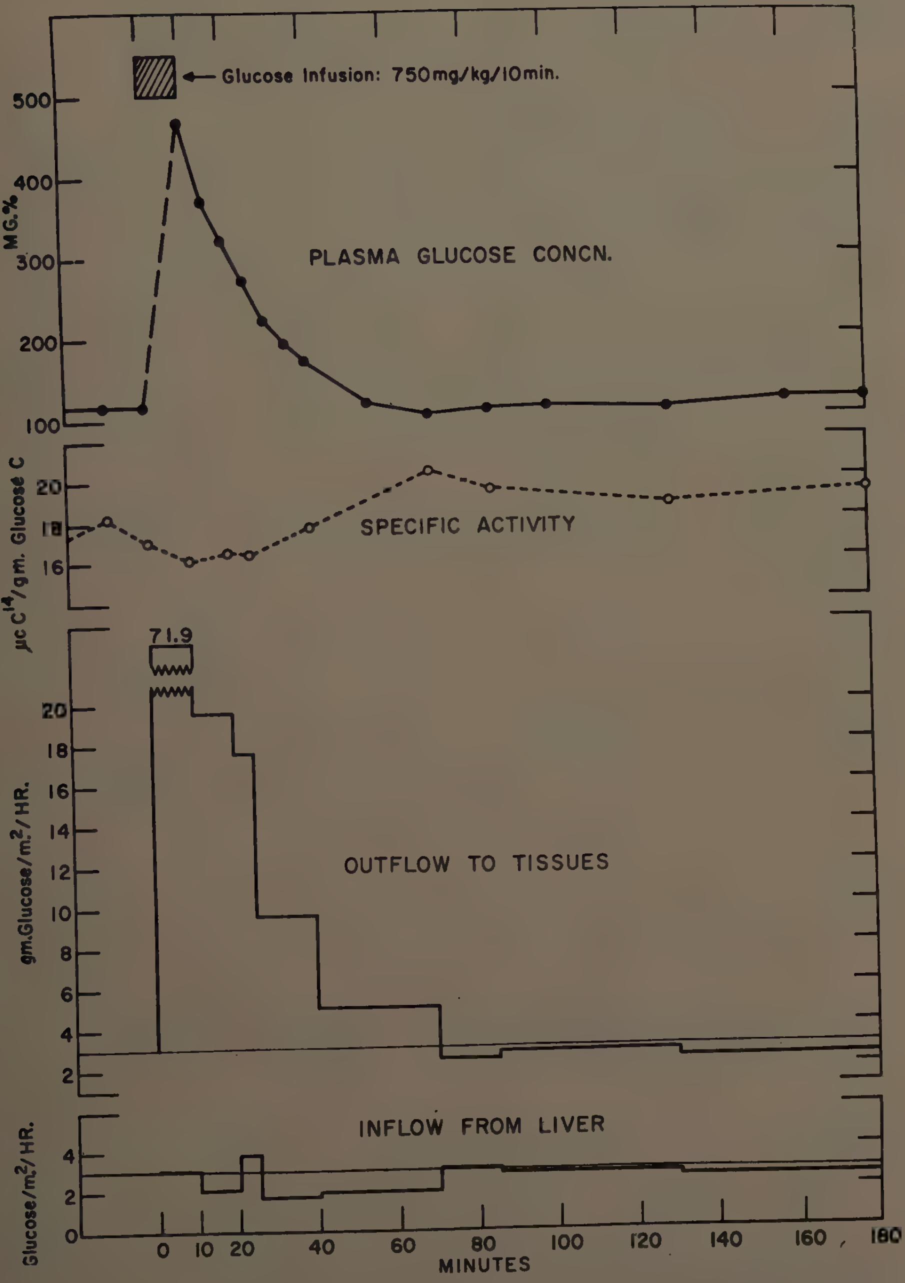

FIGURE 7. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in a normal dog given an intravenous infusion of glucose, 0.75 gm./kg. in 10 min. The glucose load was of the same specific activity as that of the plasma glucose. The trace amount of C^{14} glucose was administered throughout the entire experiment, as in FIGURE 2. The lighter reference lines are preglucose load inflow and outflow rates. The decline of the hyperglycemia was associated with a greatly accelerated outflow of plasma glucose to the tissues. The glucose inflow from liver was slightly reduced. Reproduced by permission from *Metabolism*.[28]

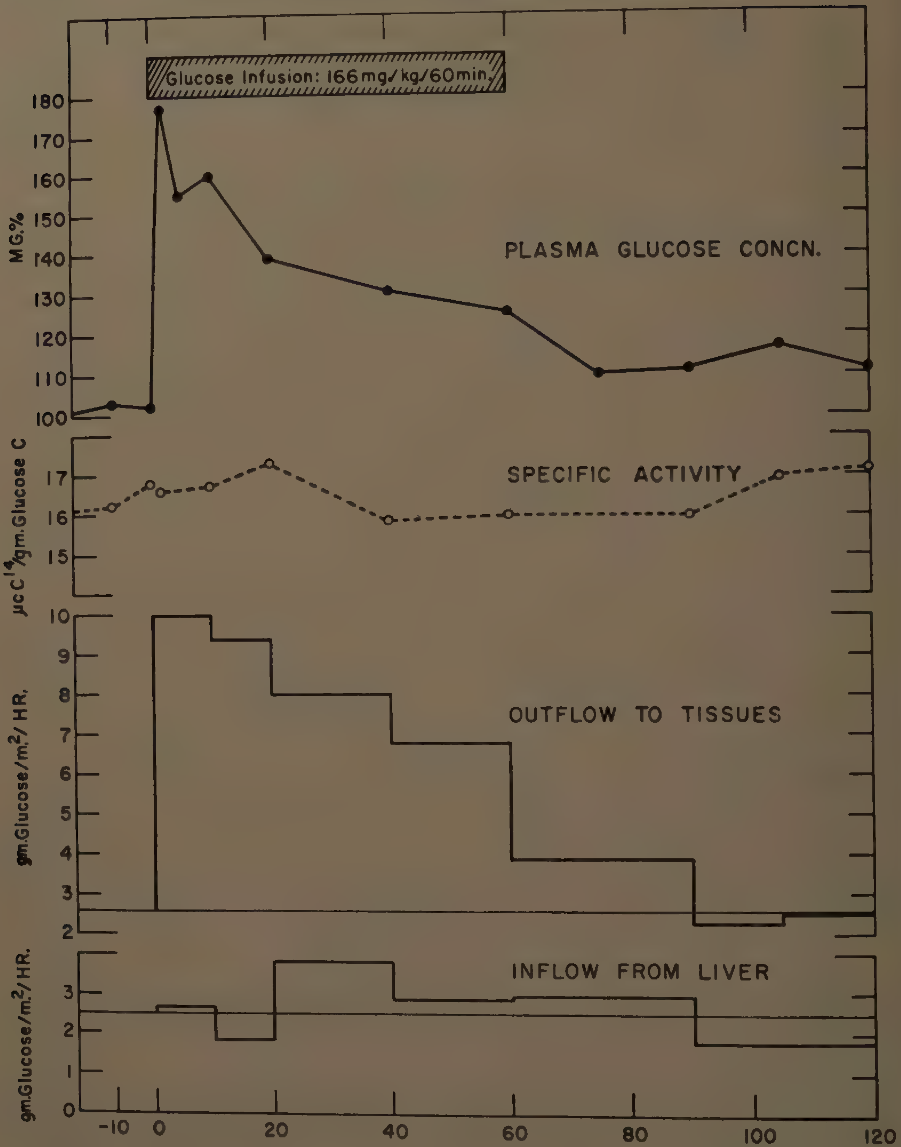

FIGURE 8. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in normal dog given an intravenous infusion of glucose (0.166 gm/kg. in 60 min.). The glucose load was of the same specific activity as that of the plasma glucose. The trace amount of C^{14} glucose was administered throughout the entire experiment, as in FIGURE 2. The lighter reference lines are preglucose load inflow and outflow rates. During the glucose load infusion the rate of plasma-glucose outflow to tissues was greatly accelerated. The glucose production, except for a brief period, was not decreased; indeed, it was somewhat elevated. Reproduced by permission from *Metabolism*.[28]

Effect of Insulin Administered by a Single Injection into a Peripheral Vein of the Hypophysectomized Dog

The preceding observations show that during the course of infusion of insulin in the normal dog there is a limitation in the increase in glucose production in response to hypoglycemia. Nevertheless, during the course of insulin infusion the rate of glucose production does increase (see FIGURES 4, 5, and 6), although not sufficiently to elevate the plasma-glucose concentration. A more marked increase in glucose production occurs following termination of insulin infusion.

Earlier studies have shown that several hormones influence glucose production.[4] In order to throw light on the interplay of the various hormones on glucose production, the effects of insulin on glucose production and utilization were studied in hypophysectomized dogs. The hypophysectomized animal is extremely sensitive to the action of insulin, as evidenced by the observation that injection of small doses of insulin results in a severe and prolonged hypoglycemia.[4] Previous studies have shown that, in the absence of the pituitary gland, insulin increases the rate of glucose utilization to a greater degree than in the normal animal, and that this is chiefly responsible for the development of the hypoglycemia.[21] As can be seen in FIGURE 9, the glucose production in the hypophysectomized dog did not increase despite the severe hypoglycemia produced by insulin. This is in marked contrast to the changes occurring in the normal animal (FIGURE 2) and explains the greatly delayed return of the plasma-glucose concentration following insulin injection to the control level in the hypophysectomized dog. Since the well-nourished hypophysectomized dog has adequate amounts of liver glycogen,[22] the reduced ability of the liver in the hypophysectomized dog to cope with the hypoglycemia must be basically due to a deficiency of anterior pituitary and adrenocortical hormones.

DISCUSSION

From the data presented in this paper it is evident that in every experiment, regardless of whether insulin was administered by a single intravenous injection, by continuous intravenous infusion, or by subcutaneous injection, the rate of glucose utilization was consistently increased during the development of hypoglycemia, during the maintenance of the hypoglycemia, and also during the period of restoration of the plasma-glucose concentration to control levels. The effects of endogenous insulin, the secretion of which was evoked by the administration of an intravenous glucose load, are also in complete harmony with the above observations, inasmuch as the high plasma-glucose levels were lowered as a result of an increased utilization of glucose. Thus, it may be stated that endogenous as well as exogenous insulin increases the utilization of glucose by the tissues.

Of the changes observed in glucose production during and following insulin administration, the most outstanding and consistent observation was the "restraining effect" of insulin on the increase in glucose production. This effect was noted in every experiment and manifested itself in the following way: during the insulin infusion, despite the severe hypoglycemia, the glucose production either stayed at the preinsulin level or, if it did increase above the preinsulin level, the increase was not sufficient to elevate the plasma-glucose concentration to control levels. A marked and adequate increase in glucose production occurred only after the insulin infusion was terminated, at which time the enhanced glucose production restored the plasma-glucose concentration to control levels.

Whether the restraining effect of insulin is a direct action of insulin on the liver or is brought about indirectly, or whether insulin interferes with the

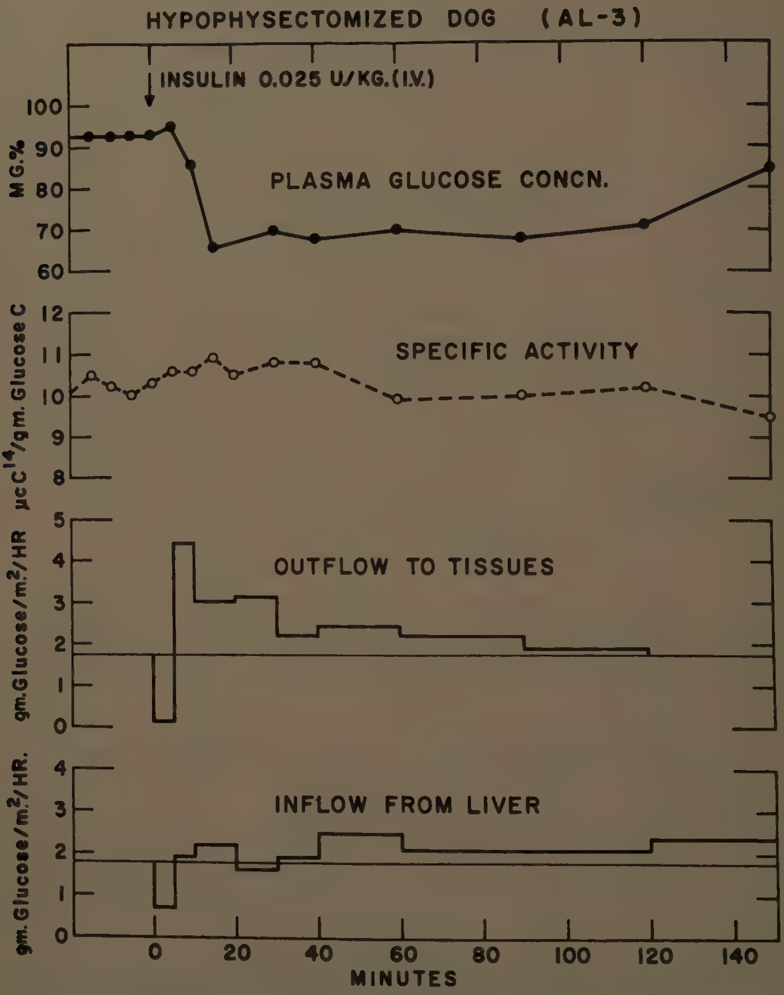

FIGURE 9. Plasma-glucose concentrations and specific activities, rates of glucose outflow into tissues, and inflow from liver in a hypophysectomized dog given a single intravenous injection of insulin (0.025 U./kg.). C^{14} glucose infusion and lighter reference lines as in FIGURE 3. C^{14} priming injection 15 μc.; infusion 0.143 μc./min. The fall in plasma-glucose concentration is due to increased outflow into tissues. Despite the hypoglycemia, the glucose inflow from the liver does not increase substantially to promptly elevate the glucose levels.

action of the blood sugar-raising hormones cannot be established at present. Recent unpublished experiments with phlorhizin have shed some light on this problem. The administration of phlorhizin to dogs in which the glucose pool was labeled as described above resulted in excretion of glucose in amounts several times greater than the size of the glucose pool of the animal. The glucose production was increased so markedly that the plasma-glucose concentration decreased only slightly or not at all. Thus, while both phlorhizin and insulin bring about an increased removal of glucose from the plasma, the glucose production is restrained only in the case of insulin. It would appear then that insulin or changes attended with the increased glucose utilization brought about by insulin are instrumental in the restraint of glucose production.

As has been shown, the ability to release glucose in response to hypoglycemia is influenced by various hormones.[21,23-25] This is evident from the observations that, in the hypophysectomized (FIGURE 9) or adrenalectomized dog,[23] the increase in glucose production following insulin-induced hypoglycemia either does not occur or is inadequate. Furthermore, these animals were shown to be insensitive to the hyperglycemic action of adrenaline[4, 22, 26] and glucagon (unpublished data). Administration of growth hormone[25] or 11-17 oxycorticosteroids[24] to the hypophysectomized dog restores the ability to increase glucose production in response to insulin-induced hypoglycemia. In addition, the steroids,[26] but not growth hormone (unpublished data), restore the sensitivity of the animal to the hyperglycemic action of adrenaline or glucagon. Thus, it is conceivable that the "restraining action" of insulin on glucose production may involve interference with the secretion or action of these blood sugar-raising hormones.

In some experiments immediately following the injection of insulin or at the beginning of the infusion of insulin, a transient rise in specific activity was noted. This effect was not present in every experiment and, when it did occur, its magnitude showed considerable variations. As stated above, this observation would indicate a decrease in glucose production that would be a contributing factor, in these experiments, to the development of the insulin hypoglycemia. However, in view of some recent findings of Shoemaker *et al.*[27] and of Mahler *et al.* elsewhere in this monograph, final judgment on this point must be postponed. These authors found, in experiments in which C^{14} glucose was administered by a single intravenous injection, that the tendency of the blood glucose to have a higher than expected specific activity after intravenous insulin injection was much more marked in the portal vein blood than in the hepatic vein blood. These authors consequently attribute this effect of injected insulin on specific activity, which ordinarily is assumed to be due to diminution of C^{12}-glucose output by the liver, to re-entry into the portal blood of C^{14} glucose of higher specific activity that has been sequestered earlier in the experiment. Whether this phenomenon contributes to the rise in specific activity following the beginning of insulin infusion in our experiments is not known at present. If future work should prove that the glucose production is indeed diminished by insulin, then the question arises whether this action of insulin is identical with the restraining effect of this hormone. If this is the case, it would appear that insulin exerts its restraining effect from the very beginning, first decreasing the glucose production and thereby contributing to the development of hypoglycemia and later preventing the release of large amounts of glucose from the liver in response to hypoglycemia.

SUMMARY AND CONCLUSIONS

The effects of exogenous and endogenous insulin on glucose production and utilization were studied in unanesthetized normal and hypophysectomized dogs, using C^{14} glucose.

Insulin, administered intravenously by single injection or continuous infusion, consistently increased the utilization of plasma glucose. The increased glucose utilization observed in the course of the insulin infusion was evident throughout the period of development and maintenance of the hypoglycemia, as well as during the return of the plasma glucose to control levels.

Similarly, plasma-glucose utilization was increased as a result of the intravenous administration of a glucose load, which presumably provoked the secretion of insulin.

The restoration of the plasma glucose concentration from the insulin-induced hypoglycemia to the preinsulin levels was invariably due to an increase in glucose inflow from the liver into the plasma. During the infusion of insulin an increase in glucose production was observed in some experiments, but it was of a limited nature and did not restore the plasma-glucose concentration to control levels. Upon termination of the insulin infusion the glucose inflow increased markedly and rapidly restored the control plasma-glucose levels. It thus appears that the increased glucose production in response to hypoglycemia is restrained during the insulin infusion.

In some experiments a diminished glucose production was observed soon after injection or at the beginning of insulin infusion, and contributed to the development of hypoglycemia.

The deficient response of the hypophysectomized dog to the insulin-induced hypoglycemia throws some light on the involvement of the pituitary and adrenal gland hormones in this phenomenon.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of G. Durandy, H. R. Bello, S. Ambrus, and C. Bjerknes in these studies.

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Discussion of the Paper

G. A. Wrenshall (*Toronto, Ont., Canada*): I am concerned about the validity of an assumption that is made in this monograph by Jacobs and Weinhouse, by Steele, and by de Bodo *et al.* Glucose spaces were determined by isotopic dilution at the start of most of the experiments reported, and they were assumed to remain constant thereafter where estimations of glucose inflow and outflow rates were made.

Is it true that the glucose spaces remain constant, as these investigators have assumed? Successive determinations by isotopic dilution of the mass of glucose with which plasma glucose intermixes rapidly, and from it of the corresponding glucose space, have been made hourly in an untreated depancreatized dog by G. Hetenyi, Jr. in Best's laboratory at the University of Toronto (FIGURE 1). These data indicate that the plasma-glucose concentration, and the above space may change slowly (6 per cent per hour) and inversely with the plasma-glucose concentration even when the glucose mass remains at or near a dynamic steady state. There is also evidence from 1 normal dog that this glucose space is more than doubled at 1 hour after the intravenous injection of insulin (1 U./kg.). The increase in the glucose space of the isolated perfused rat heart caused by insulin, and reported in this monograph by C. R. Park, appears to

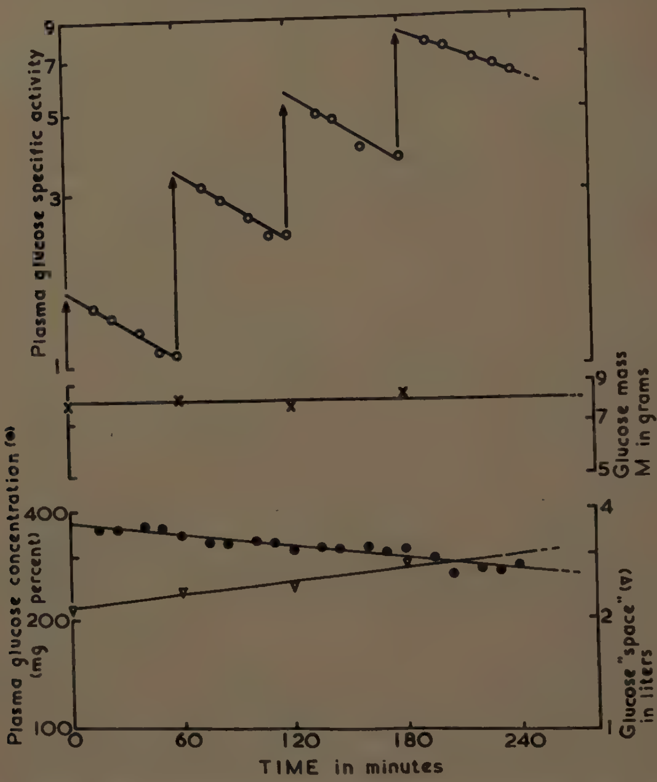

FIGURE 1. Blood plasma-glucose concentration (C), glucose mass (M), and glucose space $(V = \frac{M}{C})$ in an anesthetized, depancreatized dog 48 to 52 hours after insulin. The vertical arrows indicate the times of intravenous injection of C^{14} glucose.

support this finding. At 45 min. after the intravenous injection of tolbutamide (60 mg./kg.) the glucose space was reduced to one third of its preinjection value in a normal dog.

The mass of glucose with which labeled glucose added to the blood intermixes rapidly and its changes with time are essential for the calculation of rates of plasma-glucose transfer and accumulation.[1] These factors can be measured without reference to the glucose space that becomes involved in rate calculations where only one injection of labeled glucose is made.

To sum up, a tracer-determined glucose space in the dog appears to be subject to marked changes following the intravenous injection of either insulin or tolbutamide. Thus rates of glucose inflow and outflow, calculated on the assumption that this space remains constant after injection of these substances, are of uncertain reliability.

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HEPATIC ACTION OF INSULIN

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The role of insulin in regulating glucose metabolism by extrahepatic tissue is well recognized. It is generally accepted that the action of insulin on muscle is that of accelerating the transfer of glucose from plasma to the intracellular water.[1] The action of insulin on muscle is instantaneous and can be demonstrated in the intact animal and in various *in vitro* preparations.[2-8] This action of insulin to increase glucose utilization can be demonstrated readily with both normal and diabetic preparations. With respect to the liver, however, there is at the present time considerable doubt as to the exact mechanism of insulin action on this tissue. In contrast to muscle, liver cells are freely permeated by glucose;[9] therefore, any action of insulin to facilitate transfer of glucose must be upon some intracellular compartment. It has also been found that, while muscle from diabetic animals responds immediately to the addition of insulin, the diabetic liver is not restored to normal glucose metabolism upon the immediate administration of insulin.[10,11] Treatment of the animal with insulin over a period of several days is required to return glucose metabolism of the diabetic liver to normal.

In Vitro Studies

Chernick and Chaikoff[12] have studied the metabolism of C^{14} glucose by liver slices from normal and alloxan-diabetic rats and have concluded from their data that the impairment in glucose metabolism in the diabetic animal is due in part to an inability to phosphorylate glucose. Utilizing a similar technique, Renold *et al.*[11] were able to devise a means of calculating glucose phosphorylation by liver tissue *in vitro*. It was found that glucose phosphorylation by hepatic tissue from diabetic rats was only one fourth to one sixth as much glucose as tissue from normal rats. It was subsequently shown[11] that 12 to 24 hours of insulin action *in vivo* were required to restore glucose phosphorylation of the diabetic liver to normal.

These observations would lead one to believe that perhaps insulin has no direct action on the liver. However, there have been a number of instances in which an immediate action of insulin on hepatic tissue has been demonstrated. For example, it has been found[13] that insulin added *in vitro* to rabbit liver slices will increase the incorporation of C^{14} from labeled glucose into the tissue glycogen. It has also been demonstrated that the addition of insulin to hepatic tissue *in vitro* will accelerate the synthesis of long-chain fatty acids.[14,15] Increased lipogenesis and glycogen synthesis have been demonstrated only with tissue from normal animals, and neither effect has been uniformly reproducible.

Krahl[16] has demonstrated that insulin will accelerate the synthesis of glutathione in liver slices from diabetic rats. Glucose must be present in the system in order to demonstrate an insulin effect. It was also observed that this effect could be obtained only within a few days after the injection of alloxan.

Since the liver can produce as well as utilize glucose, it is difficult to demonstrate a net glucose uptake by this tissue. However, a decreased splanchnic-glucose output has been observed following insulin administration.[17,18] Assuming that the nonhepatic contribution to the metabolism of splanchnic area is very slight, it has been suggested that there is a direct action of insulin to increase glucose utilization and decrease glucose production by the liver. Using the isolated rat liver perfused with a medium containing 350 mg. per cent glucose, Haft and Miller[19] found that the glucose concentration of the perfusing medium increased to 600 mg. per cent within 1 hour of perfusion, and that this increase was unaffected by insulin. However, with livers from alloxan-diabetic rats, insulin produced an increased removal of glucose at these concentrations and after 1½ to 4 hours of perfusion. It was noted further that liver of ketotic animals did not respond.

In any consideration of a hepatic action of insulin one must take into account the fact that most insulin preparations contain traces of glucagon. The possibility therefore exists that a glycogenolytic response initiated by glucagon in the preparation may obscure any action of insulin. When studies are performed with the intact animal, one must also take into account the fact that any rapid fall in blood glucose induced by insulin may cause increased hepatic glycogenolysis. Whether this is due to a release of endogenous glucagon or epinephrine is not clear; however, the possibility does exist that such factors might be released and would tend to override any direct effect of insulin on hepatic tissue. These and other factors that might be involved in attempts to demonstrate a hepatic action of insulin have been more extensively reviewed by deDuve[20] and by Levine and Fritz.[21]

In Vivo Studies

Studies on hepatic tissue *in vitro* are complicated by the fact that under optimum conditions glycogen is rapidly mobilized.[22] The inevitable result is that the tissue shows a net glucose production. In an attempt to circumvent the glycogenolysis of the *in vitro* system, a number of different methods have been devised for the estimation of hepatic glucose production *in vivo*.

Hepatic venous and arterial glucose gradients. Measurements of glucose concentration in arterial and hepatic venous blood have been made by Bondy *et al.*[17] and by Sherlock and her associates.[18] Such measurements are of the splanchnic area, with obvious contributions of extrahepatic tissue. Following insulin administration, a decrease in the splanchnic glucose gradient has been observed; however, it is not possible to attribute such effects to the action of insulin on hepatic tissue.

Hepatic portal venous glucose gradients. The hepatic contribution to splanchnic-glucose output can be derived from measurements of glucose concentrations in portal venous and hepatic venous blood. Glucose gradients across the liver have been reported by Ashmore *et al.*,[23] using dogs with indwelling plastic catheters. No decrease in the portal-hepatic glucose gradient was found in such animals following injection of insulin. In an extension of these studies by Shoemaker *et al.*,[24] hepatic plasma flow was estimated by a modification of the Bromsulphalein clearance method of Bradley.[25,26] Plasma-glucose concentrations for a typical dog studied are shown in FIGURE 1. Hepatic glucose production calculated from portal-hepatic glucose gradients and plasma flow for the preinsulin period was 48 mg./min. No change was observed during the 10 min. immediately following insulin; however, a sharp increase in hepatic glucose production was observed with the onset of hypoglycemia.

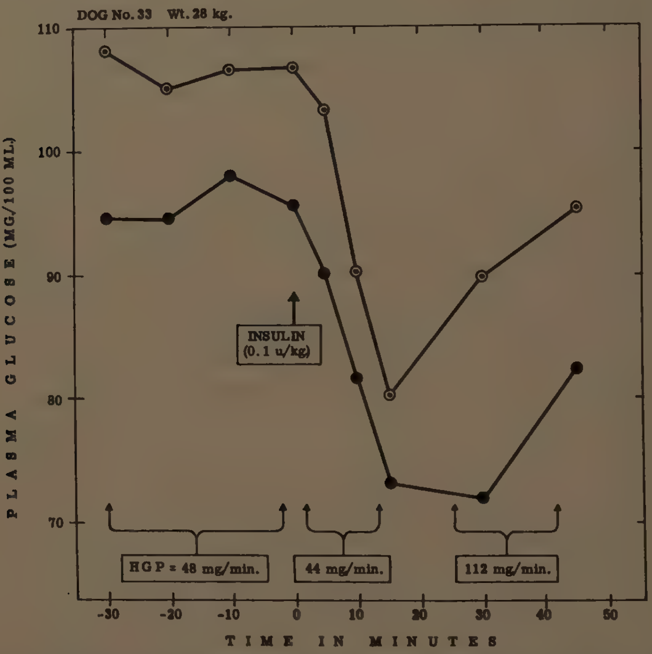

FIGURE 1. Hepatic glucose production in unanesthetized dog, estimated by hepatic catheterization. Plasma glucose in mg./100 ml. for portal (*lower curve*) and hepatic (*center curve*) venous blood has been plotted as a function of time before and after the injection of glucagon-free insulin.

In a series of 10 experiments a mean hepatic plasma flow of 31.0 ± 2.0 (S.E.) ml./kg./min. was found, and flow was not significantly altered by the administration of 0.1 to 0.2 U./kg. of glucagon-free insulin. Prior to insulin administration a mean splanchnic-glucose output of 3.9 ± 0.62 (S.E.) mg./kg./min. was observed. The mean output in the first 35 min. after insulin fell to 2.1 ± 0.65 (S.E.) mg./min. The hepatic-glucose output during the control period was 2.0 ± 0.33 mg./kg./min., and this increased to a mean of 3.9 ± 0.39 (S.E.) mg./kg./min. for the postinsulin period.[24] Although an effect of insulin to decrease splanchnic glucose production was observed, no evidence of a decrease in hepatic glucose production was obtained.

Estimation of hepatic glucose production by isotope dilution. Following intravenous injection of a tracer dose of highly active labeled glucose, the dilution of the plasma glucose can be estimated from the exponential decay of plasma-glucose specific activity.[27,28] Dunn *et al.*,[29] Tarding and Schambye,[30] and Henderson *et al.*[31] have applied this method to the estimation of hepatic glucose production of dogs following the injection of insulin. Such an approach is based on the assumption that a fall in plasma-glucose specific activity is due to dilution of the circulating glucose by unlabeled glucose produced by the liver, and that during the course of the experimental period resynthesis of labeled glucose from metabolic intermediates by the liver is negligible. Dunn *et al.*[29] have found that, following an injection of insulin, a plateau of such nature is obtained, and that for a period of about 20 min. there is no decline in the specific activity of the plasma glucose. This effect has been attributed to a direct action of insulin on the liver and has been interpreted to mean that, during the observed plateau, hepatic glucose production has completely stopped. Under essentially the same conditions, except that an anesthetized dog was used, Tarding and Schambye[30] and Henderson *et al.*[31] failed to observe any appreciable decrease in the rate of decline in specific activity of plasma glucose.

The interpretation of the changes in specific activity of blood glucose after insulin is not as simple as it might at first appear. Measurements of specific activity and concentration of glucose in portal and hepatic venous blood clearly demonstrate that the changes in glucose-specific activity following insulin cannot be attributed to any decrease in hepatic glucose production.[24] A typical experiment from the data of Shoemaker *et al.* is presented in FIGURE 2. In this figure, plasma-glucose concentration as a function of time has been plotted in the upper curve; the two lower curves are of plasma-glucose-specific activity. From the exponential decay of plasma-glucose-specific activity in the period before insulin one can calculate that hepatic glucose production in this particular dog was 118 mg./min. Calculation of hepatic glucose production from portal-hepatic glucose gradients and plasma flow during the same period gives values ranging from 104 to 138 mg./min. These 2 independent methods agree reasonably well for the control period in this particular animal.

In the period immediately following insulin it is, of course, impossible to calculate hepatic glucose production from plasma-specific activity. However, during this period the differences in portal and hepatic glucose-specific activity become more pronounced, indicating a greater dilution in passage through the liver. During this same period estimation of hepatic glucose production by analysis of portal and hepatic glucose concentrations and estimation of plasma flow was found to be essentially unchanged: from 134 to 148 mg./min.

Further evidence that isotope dilution and hepatic catheterization methods of estimation of hepatic glucose production do not always agree is presented in TABLE 1, where mean values obtained for control periods have been summarized. Values for hepatic glucose production as measured by isotope dilution are generally greater than those obtained by liver catheterization studies. In our own experiments only 1 of 4 dogs showed comparable values for hepatic glucose production when the 2 methods of measure were applied simultaneously in the same animal.

Lipscomb and Crandall[32] have used the London cannula to sample portal and hepatic blood in dogs. From their studies they have calculated a mean hepatic glucose production of 0.121 ± .012 (S.E.) gm./kg./hr. This result agrees with values that we obtained using the liver-catheterization technique. Using the isotope-dilution method, Dunn *et al.*[29] report values for hepatic glucose production in dogs ranging from 0.12 to 0.30 gm./kg./hr., which again would tend to be slightly higher than that observed by hepatic catheterization. Values ranging from 0.18 to 0.29 gm./kg./hr. have been reported from Chaikoff's labora-

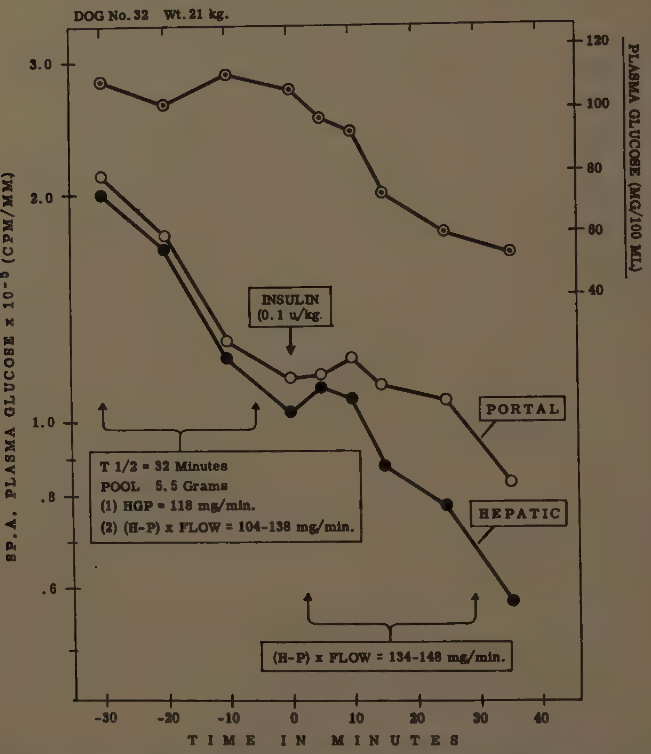

FIGURE 2. Estimation of hepatic glucose production by isotope dilution. Plasma-glucose concentration in mg./100 ml. is given in the upper curve. Specific activity of portal and hepatic plasma glucose is plotted in lower curves.

TABLE 1
ESTIMATION OF HEPATIC GLUCOSE PRODUCTION IN DOGS

Isotope dilution gm./kg./hr.	Liver catheterization gm./kg./hr.	Reference
0.35	0.16	Present study
	0.12	Lipscomb and Crandall[32]
(0.12 to 0.30)		Dunn *et al.*[29]
0.34		Feller *et al.*[27]
0.18		Searle and Chaikoff[28]
0.29*		Henderson *et al.*[31]

* Diabetic dogs.

tory.[27, 28] Henderson *et al.*,[31] using the isotope dilution method in diabetic dogs, have calculated glucose production to be of the order of 0.29 gm./kg./hr.

Steele *et al.*[33] have measured glucose production in dogs by isotope dilution, but have employed the technique of constant infusion of the labeled glucose. Under these conditions a mean glucose production of 0.15 gm./kg./hr. has been obtained from 10 experiments on 7 dogs.

Tarding and Schambye have also reported determinations of hepatic glucose production, using both the isotope-dilution and hepatic-catheterization techniques.[30] The values obtained by these authors are summarized in TABLE 2; the values from our own series are in parentheses. The data of Tarding and Schambye are reported in mg./min., and our own values have been computed on the same basis for comparison. In both the preinsulin and postinsulin periods hepatic glucose production estimations by isotope dilution are considerably higher than those obtained by liver catheterization. In either case there is no evidence of decreased hepatic glucose production after insulin.

TABLE 2
THE EFFECTS OF INSULIN ON HEPATIC GLUCOSE PRODUCTION IN DOGS*

	Method used to estimate hepatic glucose production	
	Isotope dilution	Liver catheterization
Preinsulin	80 mg./min. (118 mg./min.)	30 mg./min. (44 mg./min.)
Postinsulin	60 mg./min.	40 mg./min. (61 mg./min.)

* From the data of Tarding and Schambve.[30]

These data suggest that changes in specific activity of plasma glucose may not necessarily reflect true hepatic glucose production. The possibility exists that plasma-glucose activity may be altered by exchange of labeled and unlabeled glucose between cells and plasma without any net flow of glucose. Such an exchange could be mediated by glucose-6-phosphatase or amylo-1, 6-glucosidase.[34]

Conclusions

There is little doubt that insulin has an important role in the regulation of liver metabolism since, in the absence of the hormone, profound changes in hepatic metabolism occur. Indeed, many of the difficulties in the treatment and management of diabetes are due to liver metabolism rather than to any defect in peripheral or extrahepatic metabolism. Both diabetic acidosis and overproduction of glucose are the result of liver function.

One can readily show that there is a diminished utilization of glucose by the liver of diabetic animals. This metabolic defect is corrected by insulin, but no instantaneous action of insulin on this process has been demonstrated.

Under certain conditions it is possible to demonstrate effects of insulin on hepatic metabolism. However, the circumstances under which such effects are obtained are such that at the present time no uniform theory of action of insulin on hepatic tissue can be formulated.

There is some question as to the validity of the estimation of hepatic glucose production by the isotope-dilution method. A plateau of plasma-glucose-specific activity after insulin administration cannot be attributed to an inhibition of hepatic glucose production in the light of direct measure of glucose production by hepatic catheterization.

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FURTHER STUDIES ON THE SIGNIFICANT ROLE OF THE LIVER IN SULFONYLUREA HYPOGLYCEMIA*

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The most disturbing inconsistency between sulfonylurea-induced and insulin-induced hypoglycemia has been the repeated failure to demonstrate that the sulfonylureas induce hypoglycemia by either a direct or insulin-mediated action on glucose metabolism in the peripheral tissues. There now appears to be little question that the presence of the pancreas is an essential prerequisite for the hypoglycemic action of a sulfonylurea,[1,2] and some studies indicate that part of the action of the sulfonylureas may depend on the presence of insulin.[3-5] The degree of granulation of the beta cells appears to be related to the insulin content of the pancreas,[6,7] and the sulfonylureas have been shown to increase the quantity of islet tissue[8] and to decrease beta cell granulation.[9,10] Root has demonstrated a decrease in the insulin content of the pancreas following carbutamide administration,[11] and the results of experiments where the pancreas was directly stimulated with the sulfonylureas[12,13] support the insulinogenic action of the sulfonylureas. There are, however, only a few studies that actually show an increased insulin secretion when the insulin output is measured directly.[14] The nature of the continuous or intermittent stimulation of the pancreatic beta cells remains unknown. Other evidence has been cited to show that the sulfonylureas inhibit glucose-6-phosphatase activity,[15-17] and that they inhibit insulinase activity.[18] Other studies in depancreatized animals have demonstrated a hepatotoxic effect.[19,20] Some or all of these actions may contribute to the hypoglycemic action of the sulfonylureas in the absence of the pancreas. Nevertheless, the presence of the pancreas has proven vital to the action of the sulfonylureas; this being true, the absence of an increased peripheral "insulinlike action" following sulfonylurea administration remains a perplexing phenomenon.[21]

We have suggested previously that the mode of action of the sulfonylureas might be resolved if their administration was presumed to result in an insulin-dependent action on hepatic glucose metabolism rather than an increased insulin action in the peripheral tissues.[22] This hypothesis does not exclude the existence of a pancreatotropic action of tolbutamide, but merely transfers the emphasis on the major site of endogenous insulin action from the peripheral

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* The work reported in this paper was supported in part by the John A. Hartford Foundation, New York, N. Y., and The Upjohn Company, Kalamzaoo, Mich.

† Research Fellow of the John A. Hartford Foundation.

tissues to the liver. None of the available data on sulfonylurea hypoglycemia are inconsistent with this hypothesis, and many of the hitherto inexplicable actions of the sulfonylureas might be resolved if they were assumed to induce hypoglycemia via an increased insulin effect on hepatic glucose metabolism. Further studies undertaken in this laboratory substantiate this hypothesis.

The hypoglycemic actions of insulin and tolbutamide were studied in porta caval-shunt (Eck fistula) dogs and hepatectomized dogs. Other studies on human subjects compared the hypoglycemic actions of intraportal and intravenous injections of insulin. The results of these investigations give further support to the essential part played by the pancreas in arylsulfonylurea hypoglycemia and suggest that the major site of action of the increased insulin activity is on the liver. The removal of the liver, however, results in an increased insulin action in the peripheral tissues.

Intraportal and Peripheral Insulin on D-Xylose Disappearance

In previous publications we described the effect of tolbutamide and insulin on the disappearance of infused D-xylose in humans.[22,23] Insulin (0.1 U./kg. body weight) was injected after an intravenous loading dose of 20 gm. D-xylose and resulted in a marked increase in the disappearance rate of the D-xylose (FIGURE 1). Tolbutamide (25 mg./kg.), however, had no such effect, which suggested that tolbutamide hypoglycemia was mediated through mechanisms differing from the hypoglycemic mechanisms of intravenous insulin. Endogenous insulin must first traverse the liver before entering the general systemic circulation, and it was considered possible that this transhepatic route of insulin might result in a decreased peripheral insulin action and explain the lack of effect of tolbutamide on D-xylose disappearance. Therefore, studies were performed in human subjects undergoing intra-abdominal surgery, during which insulin was injected intraportally and its effect on D-xylose disappearance and blood glucose was measured.

Methods

Comparative studies and controls were performed in 9 male subjects during and following elective abdominal surgery. Patients undergoing cholecystectomy were considered to be ideal subjects, both from the metabolic aspects of the study and the surgical approach required for exposure of the portal vein. Immediately after entering the abdominal cavity the portal vein was identified and exposed. One hundred ml. of a 20 per cent D-xylose solution was injected intravenously into an arm vein over a 4-min. period. Twenty min. later a blood sample was drawn from a separate forearm vein, and further samples were drawn at 10-min. intervals thereafter for a total of 12 specimens or for as long as the duration of the operation would allow. The detailed technique and the methods employed for blood glucose and D-xylose determinations have already been described in previous publications.[22-24] Immediately after the fourth blood sample had been drawn, hyperglycemic-factorfree insulin (HGF-free) (0.1 U./kg.) was injected over a 2-min. period into the exposed portal vein. The same procedure was employed after the postoperative recovery period in 4 of these patients, except that the insulin in the latter studies was injected into a peripheral vein. Control studies were performed in 2 subjects at operation. One control study investigated the effect of operation alone on D-xylose disappearance, and the other the effect of operation on the D-xylose response to a peripheral vein injection of insulin.

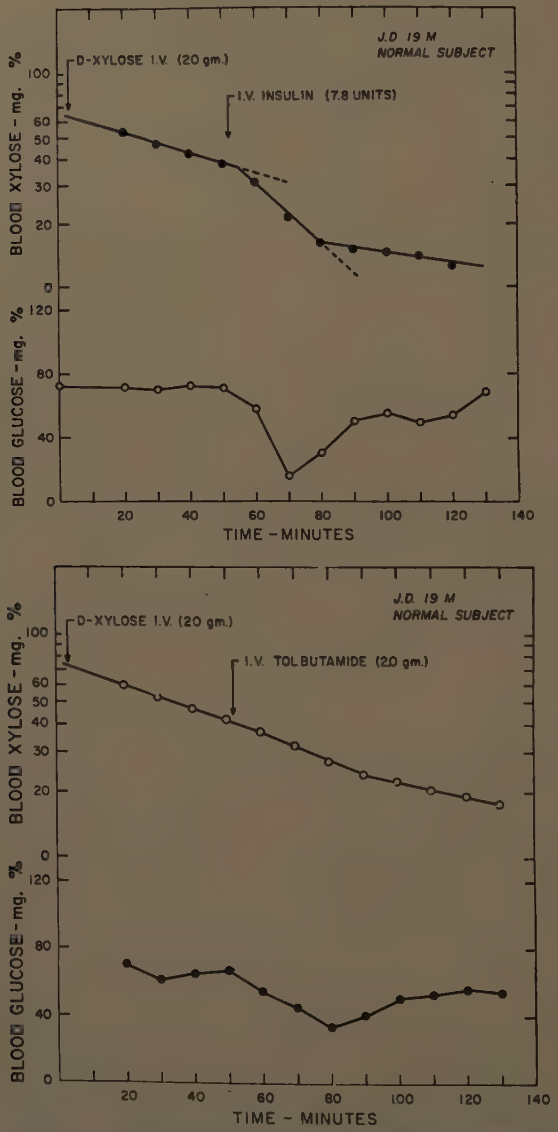

FIGURE 1. Insulin, but not tolbutamide, in man increased the disappearance rate of infused D-xylose. Both substances produced a marked hypoglycemia.

Results. In the 4 subjects studied postoperatively, insulin injected into a peripheral vein markedly increased the disappearance rate of the infused D-xylose. These results were identical to those previously described.[22,23] However, the intraportal injection of insulin resulted in only a slight effect on the disappearance rate of the infused D-xylose (FIGURE 2). During the experiments shown in this figure, blood glucose fell 65 per cent after the peripheral vein injection of insulin and 50 per cent after the intraportal injection of insulin. These results were typical for the subjects injected intraportally with insulin. This inferred that a considerable amount of the intraportally injected insulin was retained in the liver and caused a definite hypoglycemia, but the resulting decreased quantity of insulin leaving the liver was not sufficient to increase D-xylose disappearance to the extent that was seen following the injection of insulin into a peripheral vein. Control studies showed that operation did not affect the intravenous insulin action on D-xylose disappearance, and also showed that operation alone had no effect on D-xylose disappearance (FIGURE 3). For comparison, this figure also shows the results obtained during

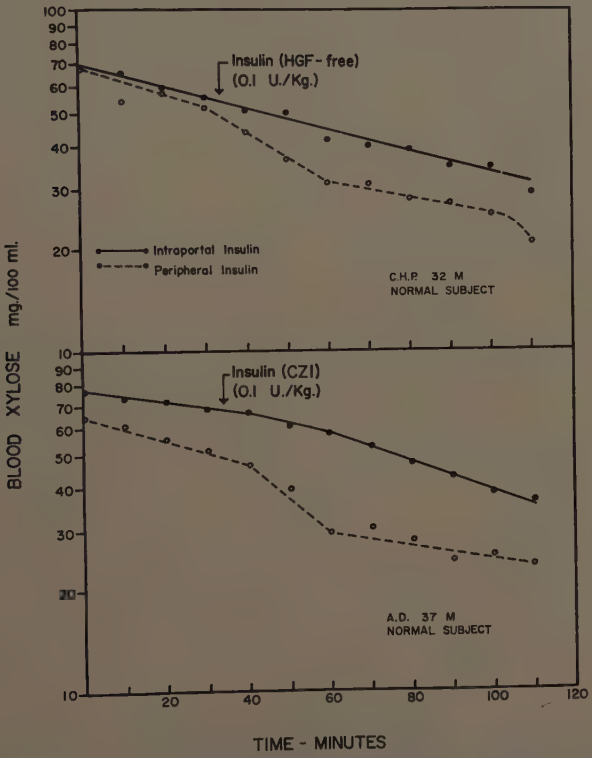

FIGURE 2. Intraportal and peripheral insulin action on pentoses. Intraportally administered insulin had little or no effect on D-xylose disappearance. Peripheral insulin consistently increased the disappearance rate of this pentose.

operation with an intraportal injection of insulin and the results obtained with an intravenous injection of insulin after operation. The implied hepatic retention of insulin could be due to either a marked and immediate hepatic retention of a considerable portion of the intraportally injected insulin or to a temporary hepatic retention followed by a slow hepatic release of insulin in amounts insufficient profoundly to affect D-xylose disappearance. That the latter hypothesis is probably the operative one is suggested by the slight effect of tolbutamide or intraportally injected insulin on D-xylose disappearance that was observed in some experiments and may be in part due to the sinusoidal character of the liver which, by the pooling of insulin in the liver, may result in a later, more gradual release of the unbound insulin.

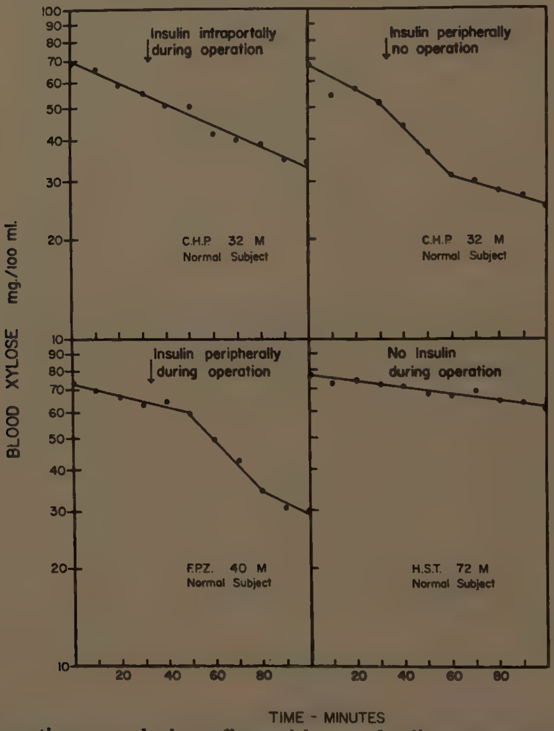

FIGURE 3. Operation per se had an effect neither on the disappearance of D-xylose nor on the action of peripherally administered insulin.

Therefore, the lack of a marked insulinlike effect on D-xylose disappearance following tolbutamide administration could be due to the transhepatic route that the increased secretion of endogenous insulin would normally take.

Tolbutamide Effects in Porta Caval-Shunt Dogs

These results led us to determine whether an initial transhepatic circulation of insulin could account completely for the differences previously observed between tolbutamide hypoglycemia and intravenous insulin hypoglycemia. In the next study peripheral glucose uptake after tolbutamide administration was measured in dogs with porta caval shunts. In these animals, endogenous insulin is secreted directly into the general venous circulation, and tolbutamide administration in them would be expected to result in an increased peripheral insulinlike action similar to that seen following an intravenous insulin infusion or injection.

Methods. Four healthy mongrel dogs weighing from 20 to 40 lb. were subjected to operation at which a side-to-side anastomosis was made between the portal vein and the inferior vena cava, and the portal vein ligatured between this anastomosis and the liver. Thus, the normal flow of endogenous insulin from the pancreas to the liver was diverted to the general systemic venous circulation. At least 2 weeks were allowed to elapse postoperatively before performing studies on these animals. Each experiment was preceded by an overnight fast and, on the following day, the dogs were anesthetized with sodium pentobarbital, and the femoral artery and vein of a hind limb were exposed on one side. At 60 or more min. after the completion of these preliminaries simultaneous arterial and venous blood samples were withdrawn by needle and syringe from the exposed femoral artery and vein. Arterial and venous blood samples were taken in this manner at 10-min. intervals for the next 20 minutes, and then tolbutamide 50 mg./kg. in 20 cc. of normal saline was injected into a separate peripheral vein over a 4-min. period. Arterial and venous blood samples were then drawn at 5, 10, 20, 30, 45, 60, 90, and 120 min. after the injection of tolbutamide. Identical experiments were performed in these same dogs, using crystalline zinc insulin (HGF-free, 0.2 U./kg.) in place of tolbutamide. The insulin was administered in 20 cc. normal saline over a 3-min. period into either a peripheral vein or a mesenteric vein. In other experiments in these same dogs a constant infusion of the same insulin preparation 0.5 U./kg. in 70 cc. normal saline was administered over a 90-min. period into either a peripheral vein or a mesenteric vein. A Harvard pump was used to maintain a constant infusion rate. During one experiment of each of the above procedures blood samples were also drawn for serum inorganic phosphate and blood pyruvic acid determinations.

Results. FIGURE 4 exemplifies the typical response in arterial and venous blood glucose to tolbutamide or insulin infusion in a porta caval-shunt dog. Immediately before the insulin infusion was discontinued, the venous blood glucose concentration had fallen by 35 per cent, and at a corresponding time following the administration of tolbutamide the venous blood glucose concentration had fallen 48 per cent. During the fall in blood glucose induced by tolbutamide the arterial glucose concentrations fell at a greater rate than the venous glucose concentrations, and this was associated with a reversal of the usual arterial-venous glucose relationship (represented in FIGURE 4 by the hatched area). The hypoglycemia accompanying insulin infused into either a peripheral or a mesenteric vein was accompanied only initially by a drop in the arterial glucose concentration, and at no time did the arterial values drop below the venous values. FIGURE 5 compares the effects of tolbutamide, insulin infusion, or insulin injection on arterial and venous blood glucose. The changes in serum phosphate and blood pyruvic acid are also shown. The

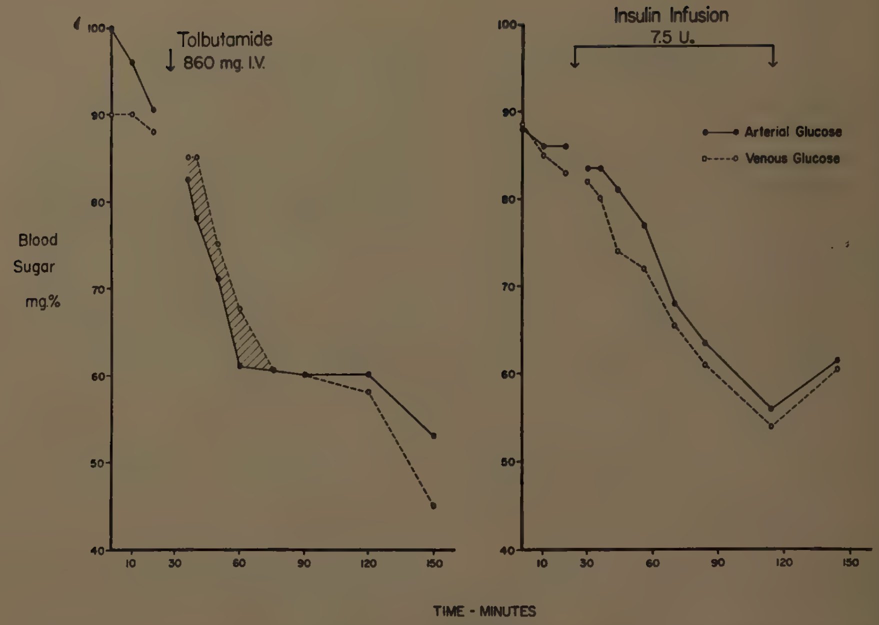

FIGURE 4. Peripheral glucose utilization in Eck fistula dogs. The crosshatched area indicates there is a reversal of the normal arterial-venous glucose relationship for the first half hour after tolbutamide when compared with an insulin infusion.

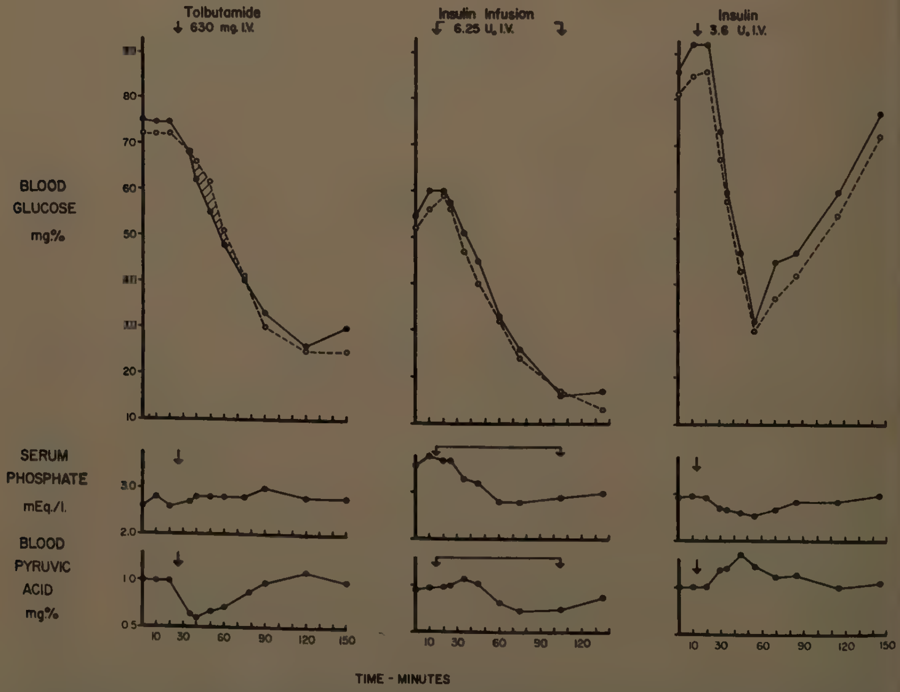

FIGURE 5. Tolbutamide and insulin effects in Eck fistula dog; tolbutamide, insulin infusion, and insulin injection are compared. Note the initially altered *A-V* glucose relationship after tolbutamide and the fall in pyruvic acid.

effects of tolbutamide and insulin infusion on arterial and venous blood glucose concentrations are identical to those shown in FIGURE 4. The hypoglycemia accompanying insulin injection differed from that due to either tolbutamide or insulin infusion in that the arterial and venous glucose concentrations initially fell at the same rate, and no alteration of the usual arterial-venous glucose relationship occurred.

The Effects of Tolbutamide on Peripheral Glucose Uptake $(A - V)/A$ in Porta Caval-Shunt Dogs

Peripheral glucose uptake can be expressed as the fraction of the arterial concentration that is removed by the limb, and this is represented by the formula $(A - V)/A$ where A represents the arterial and V the venous glucose concentration at any given moment. This fraction, $(A - V)/A$, is used as an index of peripheral blood hypolycemic activity, and insulin has been shown to increase this fraction when injected intra-arterially.[25]

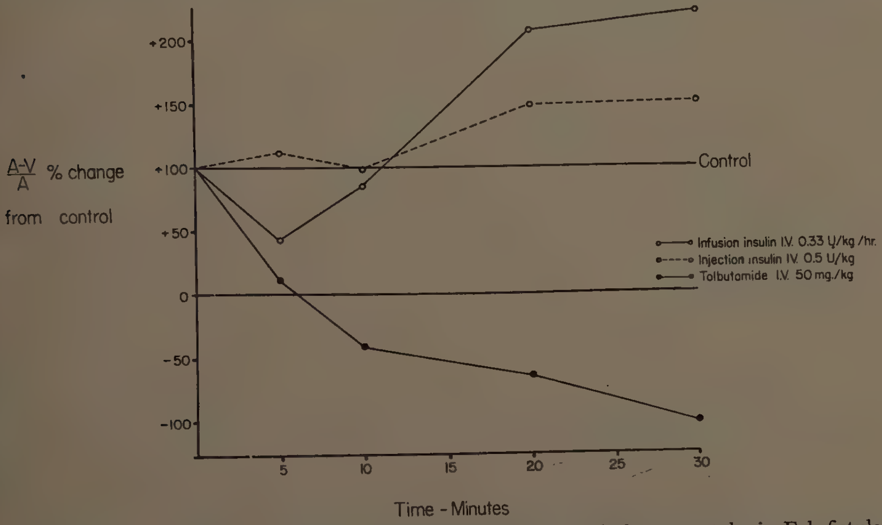

FIGURE 6. Effects of insulin and tolbutamide on peripheral glucose uptake in Eck fistula dogs. Values of $(A - V)/A$ below the zero per cent line represent an arterial glucose and concentration less than venous glucose concentration. Only tolbutamide produced a consistent progressive fall in the $(A - V)/A$ during the first 30 min. after administration.

In assessing the results of our experiments in these porta caval dogs, we reached the conclusion that the measurement of peripheral blood hypoglycemic activity as expressed by $(A - V)/A$ was valid only during the period of rapidly falling blood glucose concentration as, during the recovery phase, compensatory hyperglycemic mechanisms would play an increasingly important part in peripheral glucose uptake, and the $(A - V)/A$ fraction, therefore, would be measuring the combined activities of hypoglycemic and hyperglycemic factors. A period of 30 min. immediately following tolbutamide or insulin administration was found to contain this period of a rapid fall in glucose concentration; the changes in $(A - V)/A$ obtained during this period are shown in FIGURE 6.

This figure summarizes the results of all our studies on the effects of tolbutamide, insulin infusion, and insulin injection on peripheral glucose uptake in porta caval-shunt dogs. The vertical axis represents the percentage increase or decrease in glucose uptake from the fasting control value, and any value below the 0 per cent line represents an arterial glucose concentration less than the venous glucose concentration. Intravenous sodium tolbutamide (50 mg./kg.) resulted in a hypoglycemia and a progressive and regular decrease in the per cent uptake of glucose until the $(A - V)/A$ values became negative due to a drop in arterial glucose concentration to below the venous glucose concentration. Crystalline zinc insulin (HGF-free) 0.2 U./kg. injected into either a peripheral or a mesenteric vein in these porta caval-shunt dogs resulted initially in no significant change in glucose uptake despite a concomitant hypoglycemia. This was soon followed by an increased glucose uptake. Insulin (0.33 U./kg.) administered by constant infusion into a peripheral or mesenteric vein resulted in an initial decrease in glucose uptake, later followed by an increased glucose uptake greater than that obtained with insulin injection, even though the total amount of insulin infused at the end of 30 min. was still less than that given by injection.

It has been frequently noted that decreased or negative $(A - V)/A$ differences following tolbutamide administration occur,[26-28] and no entirely satisfactory explanation has been forthcoming to account for the apparent phenomenon of a peripheral tissue releasing glucose into the venous circulation. We feel, however, that the negative $(A - V)/A$ values observed during tolbutamide hypoglycemia in these porta caval-shunt dogs were the result of a rapid removal of glucose at sites distal to the limb. The resulting lowered blood glucose concentration returning from such a site would dilute the higher venous blood glucose concentration returning from the limbs or other unaffected sites, resulting in an eventual lowering of the arterial glucose concentration. If the resulting rate of fall in arterial blood glucose concentration was in excess of the rate of glucose utilization by the limb, then the glucose concentration in the extracellular fluid would be higher than the arterial glucose concentration, resulting in a reversal of the normal outward flow of glucose to the extracellular fluid, and glucose would re-enter the venous circulation. A decreased or negative $(A - V)/A$ fraction occurring in a limb during a developing hypoglycemia does not preclude the possibility of an increased glucose uptake by the limb, but merely states that the rate of glucose uptake by the limb was less than the rate of glucose uptake at sites other than a limb. All the foregoing considerations are true only for $(A - V)/A$ values obtained during the period of rapidly falling blood glucose concentrations.

Studies in Hepatectomized Dogs

The next step taken was to ascertain whether the liver was causing the narrowing and reversal of the $(A - V)/A$ values by the mechanisms previously discussed; that is, by a decrease in hepatic glucose output and/or an increase in hepatic glucose uptake. A modified form of hepatectomy was devised and developed by one of us (E. J. M.). The technical details of this operation will be the subject of a separate article.

Methods. Hepatectomized dogs received an intravenous injection of tolbutamide (50 or 100 mg./kg.) during a glucose infusion administered into a jugular vein at a rate of 250 mg./kg./hour. This infusion rate was selected because these animals did not develop hypoglycemia at this rate and, by supplying 80 per cent of the resting daily caloric requirement, it presumably, therefore, would not stimulate to any great degree the pancreatic secretion of insulin. Simultaneous arterial and venous samples were drawn from a femoral artery and vein by syringe and needle at 15-min. intervals starting one half-hour after the completion of the hepatectomy. Venous blood samples were analyzed immediately for glucose concentration and, when a static blood glucose level had been present for 1 or more hours, tolbutamide (50 or 100 mg./kg. in 20 ml. of normal saline) was injected over a 4-min. period into a peripheral vein. Simultaneous arterial and venous blood samples were then drawn at 15-min. intervals for an additional 4 to 6 hours. In 2 such experiments samples were also drawn from an indwelling catheter in a jugular vein for serum potassium and inorganic phosphate determinations.

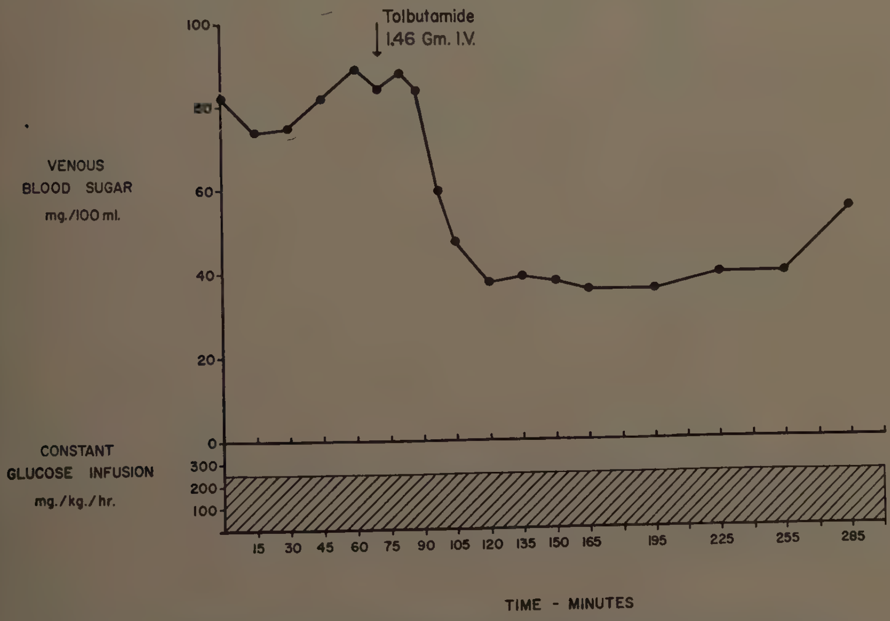

FIGURE 7. Effects of intravenous tolbutamide in blood sugar in the hepatectomized dog. A prompt fall in blood sugar followed tolbutamide administration in the liverless dog.

Results. The effect of intravenous tolbutamide (50 mg./kg.) on the venous blood glucose concentrations of a hepatectomized dog is shown in FIGURE 7. Note that, apart from minor fluctuations, the blood glucose concentration had been stable for 75 min. prior to the injection of tolbutamide. After tolbutamide injection there was a dramatic fall in blood glucose concentrations that eventually dropped to less than 50 per cent of the control values. Figure 8 shows the arterial and venous blood glucose responses to tolbutamide. Serum potassium and serum inorganic phosphate concentrations are also shown in the same figure. Note that the blood glucose concentrations were again stable prior to the injection of tolbutamide. Tolbutamide injected intravenously during the period of stable blood glucose concentrations resulted in a definite hypoglycemia. The developing hypoglycemia was not accompanied by the reversal in arterial and venous blood glucose concentrations that was seen following tolbutamide injection in porta caval-shunt dogs. Determinations of the changes in $(A - V)/A$ during the period of developing hypoglycemia revealed an increased glucose uptake comparable to that obtained with an insulin injection in a port caval-shunt dog. Similar results were obtained in

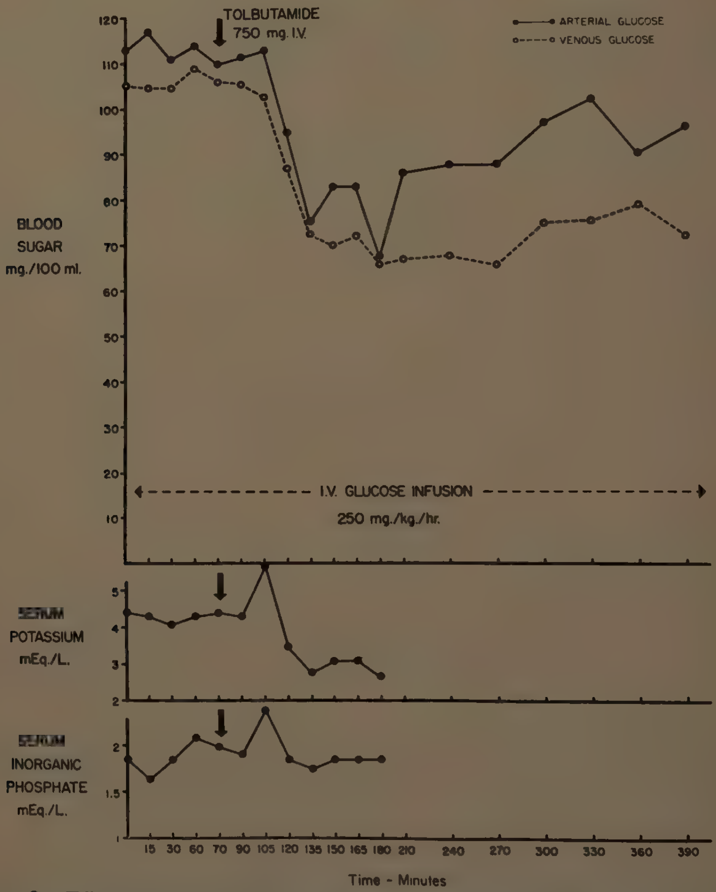

Figure 8. Effects of intravenous tolbutamide in a hepatectomized dog. The hepatectomized dog showed an increased $A - V$ difference after tolbutamide similar to that of insulin given to normal or hepatectomized animals.

the 6 hepatectomized dogs that have been studied so far. The accompanying changes in serum potassium and serum inorganic phosphate are shown in FIGURE 8; the fall in serum potassium seems to indicate an increased insulinlike activity following tolbutamide administration. This did not occur in the porta caval-shunt dogs. These results demonstrate that, in the absence of the liver, tolbutamide administration results in a hypoglycemia with essentially the same characteristics as that obtained with insulin in porta caval-shunt and normal dogs. These results are therefore consistent with the suggested insulinogenic action of the sulfonylureas and conclusively proved that the sulfonylureas are active hypoglycemic agents even in the absence of the liver.

Serum Amino Nitrogen Studies in Man

Evidence is accumulating that insulin plays a role in the metabolism of amino acids. The plasma level of amino acids is elevated in patients in diabetic acidosis.[29] Luck and Morse[30] have shown that the administration of insulin causes a decrease in plasma amino acids in normal animals. It has been suggested that the decrease in amino nitrogen is mediated through epinephrine for, in the adrenal demedullated rabbits, this fall in amino nitrogen with insulin was not observed.[31] However, Frame and Russell were unable to confirm these observations,[32] and they also noted that the administration of insulin in eviscerated rats depresses the rate of increase of plasma amino nitrogen that consistently occurs following this operative procedure. This observation suggests a peripheral effect of insulin on amino acid metabolism, but it is not known whether insulin promotes the synthesis of tissue protein from plasma amino acids or inhibits the formation of amino acids.

Tolbutamide may influence the formation of protein by the liver. Pretreatment of rats with tolbutamide was found to increase markedly the incorporation of C^{14}-glycine into protein in liver slices.[33] DeMeutter *et al.*[34] have recently found that tolbutamide injected intravenously into human subjects causes a decrease in serum alpha-amino acids, and that this decrease was similar to that produced by insulin in normal human subjects and diabetics. Our observations confirm these workers' findings.

Methods. The subjects used in these studies were 4 normal male volunteers, aged 22 to 26 years, and 3 diabetics, 2 of whom were of the obese type, aged 44 and 56 respectively, and 1 a labile diabetic aged 33 years. All subjects were fasted overnight prior to the test procedures and, in the diabetics, crystalline zinc was substituted when necessary for the long-acting insulin during the preceding 24-hour period. Tolbutamide (25 mg./kg.) or crystalline zinc insulin (0.8 to .15 U./kg.) was injected intravenously the morning of the test. Venous blood specimens were taken at 15-, 30-, or 60-min. intervals for 3 to 4 hours. Serum amino nitrogen determinations were performed by the Albanese method.[35]

Results. FIGURE 9 shows that insulin produced a significant decrease in serum amino nitrogen in both the normal volunteers and diabetics. In the normals there was a 17 per cent decrease from the initial level and, in the diabetics, a fall of 21 per cent at 3 hours. Insulin and tolbutamide produced a fall in blood sugar that was prompt and significant (FIGURE 10). This fall amounted to 71 per cent with insulin and 38 per cent with tolbutamide in the normals. The diabetics were less responsive for, with insulin, there was only a 28 per cent decrease even with a larger dose of insulin; with tolbutamide, the maximum fall of 32 per cent occurred at 3 hours.

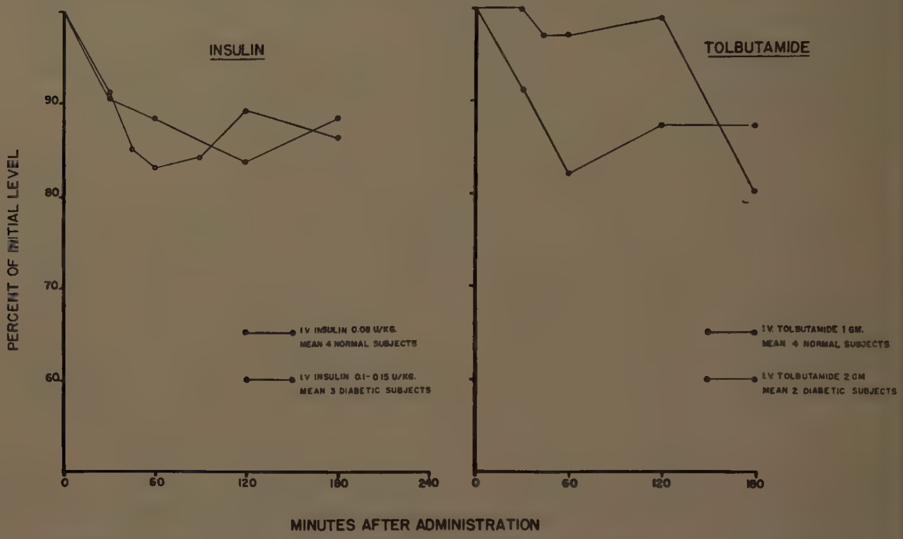

FIGURE 9. Serum amino nitrogen following insulin and tolbutamide. Both insulin and tolbutamide caused a lowering of serum amino nitrogen in normal and diabetic subjects.

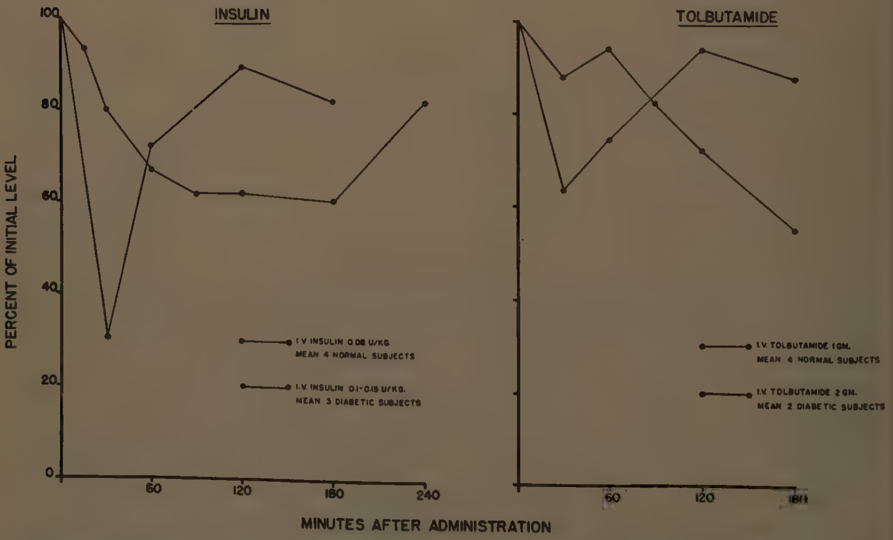

FIGURE 10. Blood glucose following insulin and tolbutamide. These graphs show the blood sugar responses to tolbutamide and insulin given to the same subjects as in FIGURE 9.

Discussion

The results presented in the foregoing studies were designed to determine further whether the hepatic action of the sulfonylurea compounds proposed in previous publications could be substantiated.[22,23] While the results are consistent with such a hypothesis, they do not explain as yet the precise mechanism whereby this hepatic result is effected. However, from our results certain possible mechanisms are suggested.

Interpretation of results of intraportal insulin studies. It has been shown that, unlike insulin, tolbutamide does not increase the disappearance rate of infused D-xylose.[22] Since the premise that tolbutamide affects pancreatic insulin output is, in our judgment, well established, and since this insulin traverses the liver before reaching the peripheral circulation, the question arises as to whether the reason for the lack of effect of tolbutamide on D-xylose might be due to an ineffective amount of insulin reaching the peripheral circulation. The very minor effect on D-xylose disappearance rate after intraportal insulin administration suggests that the liver has retained a large measure of the injected insulin or that insulin is bound by the liver and then slowly released in amounts insufficient to produce a marked effect on D-xylose. The possibility that insulin is altered by the liver and is thereby no longer effective in the periphery seems most unlikely. Kaplan and Madison[36] have made observations on the hepatic binding of insulin-I^{131} injected into the portal vein of human subjects undergoing abdominal operation. When the subjects were given no glucose prior to the intraportal injection of insulin, 54 per cent of the injected tagged insulin was bound to the liver in a single transhepatic circulation. With increasing loads of glucose the binding declined progressively from 38 per cent to 7.8 per cent. Thus, hepatic binding of insulin does occur and may explain our failure to observe any marked effect on D-xylose disappearance following intraportal insulin. Operation alone does not play a role, since peripherally administered insulin during operation causes a normal increase in D-xylose disappearance, and the studies of Skinner and Madison[37] in rats indicate that during operative stress the hepatic binding of insulin is actually lessened.

In summary, both intraportally administered insulin and tolbutamide have an insignificant effect on D-xylose disappearance. This suggests a hepatic binding of insulin but, as yet, does not indicate whether this binding is increased by tolbutamide, although there is indirect evidence indicating that this may be the case.

Interpretation of results of porta caval-shunt studies. The use of the arterial-venous glucose difference as a measure of peripheral glucose utilization was suggested by Somogyi.[38] Elrick[39] has advocated the use of the ratio (*arterial glucose − venous glucose*)/*arterial* glucose as a measure of peripheral glucose uptake. The precise measurement of peripheral glucose uptake should also include blood flow rates for the part under study[40] but, provided that there is no interference with blood flow rates such as would occur with the use of an indwelling catheter, this rate can be presumed to be constant under stable conditions, and the use of the fraction $(A - V)/A$ can be used as a measure of glucose uptake. Bell and Burns[25] have shown that the intra-arterial injection of insulin results in a wider $A - V$ difference in the limb under study than in the contralateral limb. Craig *et al.*[28] have shown that the intra-arterial injection of tolbutamide has no insulinlike action. Employing the arteriovenous glucose difference as an index of insulin activity in acute studies, Purnell *et al.*,[26] using direct arterial and venous blood glucose determination, have shown that tolbutamide does not affect the peripheral utilization of glucose, but Goetz *et al.*,[41] using capillary instead of arterial blood, obtained an increased glucose uptake with tolbutamide. This finding was later supported by the studies of Madison and Unger,[42] who used direct arterial-venous sampling. However, these acute studies, for the most part, measured peripheral glucose utilization over the whole experimental period. We have, however, limited the interpretation of our $(A - V)/A$ data in porta caval-shunt dogs to a period of 30 min. following tolbutamide, as this limit was found to contain the period of most rapid fall in blood glucose and, we feel, represents the initial hypoglycemic action of the sulfonylureas without the modifying influence of any significantly acting compensatory hyperglycemic factors.

It can be seen from our results that the initial tolbutamide hypoglycemia in porta caval-shunt dogs is not the result of an enhanced glucose uptake in the periphery, although an increased peripheral glucose uptake did occur after 30 to 60 min. This was at a time when the blood glucose had ceased to fall at its previous rate and had reached, or was reaching, a static level. The negative $(A - V)/A$ values obtained during the period of falling glucose values suggest that an increased glucose uptake at some central or visceral site causes the initial hypoglycemia after tolbutamide administration. That the hypoglycemic mechanism involves the liver has been shown in the subsequent experiments in hepatectomized dogs and is supported by the work of other investigators using different techniques.[43-47] Madison *et al.*,[48,49] using insulin infusions in porta caval-shunt and normal dogs, have demonstrated that the resulting hypoglycemia is due to a decreased glucose output from the liver that was also associated with an increased glucose uptake by the liver. In our present studies, using higher rates of insulin infusion than those used by Madison and his co-workers, we demonstrated an initial hepatic effect soon followed by a marked peripheral action. This difference was presumably due to an overloading of the hepatic insulin-retaining mechanism with resulting increased peripheral effects. Our studies also demonstrated the fact that the reduction in glucose output from the liver is not due to the normal physiological route taken by endogenous insulin, but is presumably dependent on a slow release of insulin only.

Interpretation of results on hepatectomized dogs. There are now many studies demonstrating that the hypoglycemic action of the sulfonylureas is independent of the presence of the liver.[44,46] None of these studies, however, determined whether sulfonylurea-induced hypoglycemia in hepatectomized animals had features different from the sulfonylurea-induced hypoglycemia in intact animals. As previously noted in these and other studies, sulfonylurea hypoglycemia has features different from insulin hypoglycemia when insulin is given by injection into a peripheral vein.[22,50-52] It is therefore of interest to note that tolbutamide administration in the absence of the liver resulted in the appearance of an increased peripheral glucose uptake, an effect that could not be obtained when the liver was present. The demonstration of an increased peripheral glucose uptake in the hepatectomized animal and the lack of an increased peripheral glucose uptake in the face of hypoglycemia in the porta caval-shunt animal points to the liver as being the preferential site of glucose uptake following tolbutamide administration. Furthermore, the demonstration of an increased peripheral insulinlike action following tolbutamide in the hepatectomized animal supports the view held by many investigators that sulfonylureas stimulate endogenous insulin secretion. Finally, it has been shown in these studies that, although the physiological initial transhepatic circulation of insulin may be a factor in producing the special features accompanying sulfonylurea-induced hypoglycemia, it is not an essential factor, and the relative degrees of hepatic and peripheral effects also can be modified by decreasing or increasing the rate of insulin administration, a high rate resulting in a combined hepatic-peripheral insulin effect with the peripheral insulin effect dominant, and a low rate of insulin administration resulting in a decrease in the peripheral insulin effect so that the hepatic insulin effect becomes dominant.

Interpretation of serum amino-nitrogen studies. It has been shown that insulin does reduce serum amino-nitrogen, and that this effect is both hepatic and peripheral. The results presented in this paper indicate that both insulin and tolbutamide lower the serum amino nitrogen. Bornstein[53] has shown that there is depression of gluconeogenesis with tolbutamide, and this is offered as one explanation for the reduction in blood sugar. Recant and Fischer[33] showed that pretreatment of rats with tolbutamide caused a greater incorporation of glycine into protein by their livers.

Our results correspond closely to those of DeMeutter,[34] and are interpreted as indicating that gluconeogenesis is not blocked by tolbutamide and that the liver may be the primary site of amino acid uptake following tolbutamide administration. Further studies are indicated in order to elucidate whether the liver is the only site of this effect.

Conclusions

Considerable controversy exists concerning the sites involved in sulfonylurea hypoglycemia. The evidence supporting a peripheral site and little or no participation by the liver conflicts with other evidence supporting a hepatic site with little or no peripheral participation. In the present studies, however, we were concerned only with the role of the liver in the initiation of the hypoglycemic response to the sulfonylureas. These studies complement the previous observations from this laboratory on the role of the liver in sulfonylurea hypoglycemia, and the results support our previous contention that the liver is the predominant site participating in the initiation of sulfonylurea hypoglycemia. The porta caval-shunt dogs were originally thought to be ideal preparations for the demonstration of an increased peripheral insulin action following sulfonylurea administration. The unexpected lack of an increased peripheral insulin action and the subsequent demonstration of a predominant hepatic insulin action following sulfonylurea administration in these animals led us to conclude that the initial transhepatic circulation of endogenously secreted insulin was not the only factor responsible for the occurrence of the predominant hepatic action. The relatively long survival period of the dogs hepatectomized by a modified procedure enabled us to demonstrate positive changes in peripheral glucose uptake $(A - V)/A$ and other biochemical parameters that support the insulinogenic effect of the sulfonylureas. Furthermore, upon removing the liver we were able to demonstrate a peripheral initiation of the hypoglycemic responses to sulfonylurea administration.

No conclusions can be drawn from these studies on the degree of peripheral participation in the initiation of sulfonylurea hypoglycemia in the intact or porta caval preparations except to state that, if the periphery does participate in the initial hypoglycemic phase, then this participation must be less in extent than in the liver. The maintenance of the hypoglycemic levels after sulfonylurea administration was due to an increased peripheral participation, but the degree to which the liver participated in this later phase could not be determined from these results. These results also suggest that the rate of secretion of endogenous insulin controls the relative contributions of the liver or the periphery to the maintenance of hypoglycemia, but that the initiation of the hypoglycemia is due to the predominant but not exclusive participation of the liver Indirect evidence has been presented to indicate that the nature of the hepatic participation is a net increase in glucose uptake by the liver and is, therefore, not due soley to an inhibition of glucose release from the liver cells.

As yet unanswered by these studies are the following possibilities among others: Is the hepatic effect due solely to the rate of endogenous insulin secretion after sulfonylurea stimulation? Is there an increased hepatic binding of insulin? Is the marked hepatic effect due to a direct action of the sulfonylureas on the liver cells, making them hyperresponsive to insulin? Has endogenous insulin been altered in some way so that it has an increased hepatic effect? These studies, therefore, reveal the need for further investigations into the factors responsible for the predominant participation of the liver in the initiation of the hypoglycemic response to sulfonylurea administration.

Acknowledgments

Grateful appreciation is expressed to Thomas Casey who participated in the early phases of this work, to Elizabeth Sheldon, for laboratory help, and to Carol Cote for secretarial and art work.

The tolbutamide used in these studies was supplied by C. J. O'Donovan, The Upjohn Company, Kalamazoo, Mich. The glucagon-free insulin was made available by W. R. Kirtley, The Lilly Research Laboratories, Indianapolis, Ind.

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CLINICAL AND EXPERIMENTAL STUDIES OF INSULIN SECRETION FOLLOWING TOLBUTAMIDE AND METAHEXAMIDE ADMINISTRATION

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An increasing body of evidence supporting the concept that liberation of pancreatic insulin represents an important action of the sulfonylurea drugs has accumulated since publication of the monograph *The Effects of the Sulfonylureas and Related Compounds in Experimental Diabetes* in 1957 by The New York Academy of Sciences.[1]

As is apparent from the topics of a number of the papers included in the present monograph, the question is no longer whether or not stimulation of endogenous insulin secretion occurs following the administration of tolbutamide, but whether the different findings still observed after treatment with sulfonylureas or with exogenous insulin can be ascribed to the specific nature of tolbutamide-released pancreatic insulin or whether they should better be attributed to some other factor.[2]

One point, however, should be given special attention in the present phase of interest in endogenous insulin discharged from the pancreas after the administration of tolbutamide. In spite of the recent data on hepatic trapping of pancreatic insulin, the question remains as to why many investigators have failed to demonstrate increased plasma insulin levels in man following tolbutamide therapy. It is not likely that all the pancreatic insulin remains in the liver, therefore not appearing in peripheral circulation. It is true that only recently we ourselves also tried to offer a positive solution to the problem of the failure of increases in peripheral insulin levels in man on this basis.[3] However, even in those experiments performed in dogs in which the plasma insulin levels were determined in the portal and the femoral venous blood simultaneously after a single dose of tolbutamide, the increases in plasma insulin in the portal vein were found in 2 animals only and did not appear in the peripheral circulation. In the other experiments a significant elevation of plasma insulin-like activity was established in both portal and peripheral circulations following the injection of tolbutamide.

Consequently, it appeared feasible to reinvestigate the whole problem of peripheral blood insulin levels in man following the administration of a single dose of tolbutamide by using the same bioassay procedure for measuring insulin in blood (the rat adipose tissue method[4]), which was already found to be reliable in experiments on dogs.[3] This project appeared even more justified when a new compound of the sulfonylurea family, metahexamide, was being investigated. In view of the fact that a very low dose of this drug is effective in producing hypoglycemia in normal humans and elderly diabetics, information regarding its insulin-stimulating capacity seemed particularly desirable.

The studies presented here were undertaken in humans as well as in animals. With the former, major attention was given to the possible variations of blood insulin levels in the various groups of patients submitted to the test. In the studies carried out in rats, special consideration was given to the expected differences in insulin levels in portal and peripheral venous blood before and after a single dose of the blood sugar-lowering drugs had been administered and to the determination of pancreatic insulin at the end of the experiments. In both studies the effect of tolbutamide in respect to insulin stimulation was compared with that of metahexamide, each human subject serving as his own control.

Studies in Men

Subjects and methods. The human subjects for these studies were 5 nondiabetic persons of both sexes, ranging in age from 19 to 35 years, 5 elderly persons, and 4 juvenile diabetics. In the elderly diabetics (2 males and 3 females aged from 52 to 71 years) the diabetes was of the mild or stable type, requiring little or no insulin. None of the patients had been diabetic for more than 48 months, and 2 patients had been treated with tolbutamide tablets successfully for 1 and 2 months, respectively, prior to the present investigation. This therapy had been stopped in each case 24 hours before carrying out the sulfonylurea-response test.

In the 4 juvenile diabetics, 1 male and 3 females, the metabolic disorder, diagnosed within the first 2 decades of life in each case, had existed at the time of the study for an average period of 13 years. The mean requirement of exogenous insulin amounted to 60 units of a long-acting preparation, the last injection of which was given 24 hours before the administration of the single dose of the antidiabetic drug.

In addition to these studies on groups of patients, an individual subject, a 32-year-old female suffering from a pancreatic islet-cell tumor that was proved later at operation, was subjected to the same investigative procedure.

All studies were carried out after an overnight fast. After venous blood samples for determinations of fasting blood sugar and insulin had been obtained at zero time, 25 mg. of tolbutamide/kg. body weight were given in a rapid injection intravenously, and blood was obtained 30, 60, and 180 min. after the administration of the drug. After an interval of at least 3 days the effect of metahexamide was investigated in the same manner and in the same patient by administering a single intravenous dose of 2.5 mg. metahexamide/kg. body weight.

Blood glucose was measured according to the method of Hagedorn and Jensen.[5] The insulin was assayed by using the method of Martin *et al.*[4] This bioassay is based, as mentioned above, upon the oxidation of glucose-1-C^{14} to $C^{14}O_2$ by the rat epididymal adipose tissue. It has been slightly modified in the present studies by using diluted serum (1:2) instead of undiluted plasma, since (1) no significant difference in insulinlike activity was found between plasma and serum, and (2) dilution of serum yielded higher and more constant insulin concentrations.[6] This dilution phenomenon, originally described in insulin assays using the rat hemidiaphragm,[7] is explained by the fact that the activity of insulin-inhibiting substances in serum diminishes more rapidly with dilution than the effect of the serum insulin.[8] Each measurement was carried out in triplicate; that is, each unknown was assayed simultaneously with the epididymal adipose tissue of 3 rats. Eight aliquots of 1 serum of a normal subject measured in 1 procedure showed variations in insulinlike activity of ± 40 per cent. The index of precision (lambda) for dose response curves from 62 to 1000 μU./ml. has been 0.29 (FIGURE 1). Normal fasting levels ranged from 135 μU./ml. to 680 μU./ml. in 15 subjects.

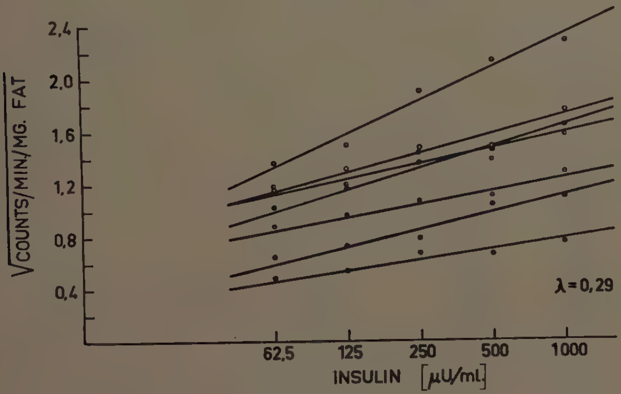

FIGURE 1. The effect of insulin on $C^{14}O_2$ production from glucose-1-C^{14}.

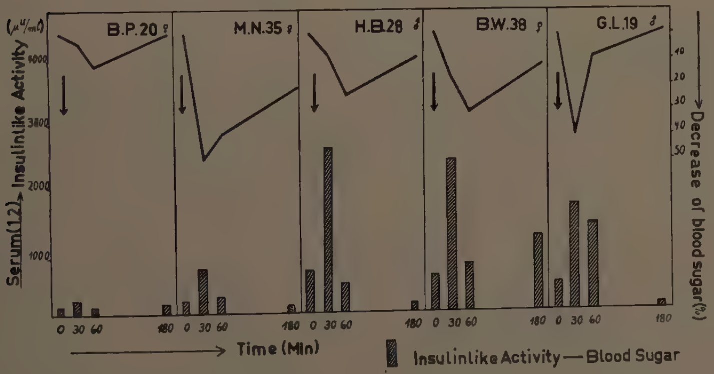

FIGURE 2. The response of normal subjects to a single dose (25 mg./kg., intravenously) of tolbutamide (Orinase).

Results

Serum insulinlike activity in 5 normal subjects before and after the administration of tolbutamide and metahexamide, respectively. In FIGURE 2 are shown the results obtained by determining the serum insulinlike activity and the blood sugar concentration before and after the intravenous administration of 25 mg. tolbutamide/kg. body weight in 5 subjects free of metabolic disorders.

In 4 of the 5 subjects increases in serum insulinlike activity were observed 30 min. after tolbutamide, that is, approximately at or shortly before the maximum decrease in blood sugar concentration. One hour after injection in 3 of the 4 subjects and 180 min. following the tolbutamide in all cases, the insulinlike activity in the serum as well as the blood sugar had returned within range of the initial values.

It thus appears that a correlation exists between the increase in serum insulinlike activity and the fall in blood sugar, expressed as a per cent decrease. However, this correlation does not appear to depend upon the absolute rise in insulin activity in the serum, since approximately the same fall in blood sugar has been observed in subjects in whom the insulin levels increased to more than 2000 μU. (Case Nos. 3 and 4) as in others where only 700 μU./ml. serum have been measured as a maximum (Case No. 2).

The changes in serum insulinlike activity and blood sugar concentration induced by the injection of 2.5 mg. metahexamide/kg. of body weight in the same 5 subjects are demonstrated in FIGURE 3. These results were similar to, although less marked than, those obtained with tolbutamide.

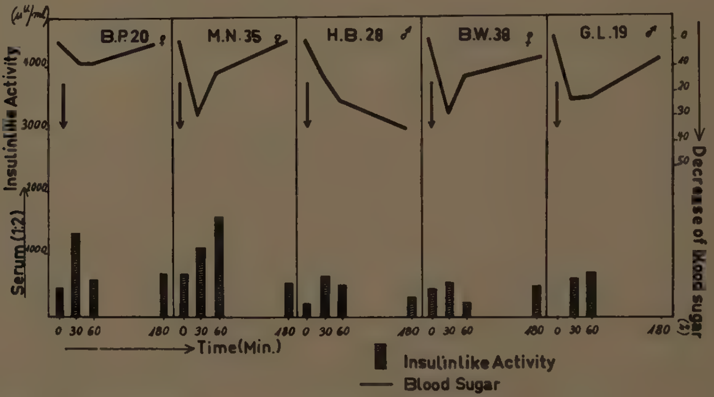

FIGURE 3. The response of normal subjects to a single dose (2.5 mg./kg., intravenously) of metahexamide.

This effect is illustrated further in FIGURE 4, which compares only the serum insulinlike activity levels obtained after tolbutamide with those observed after metahexamide in the 5 healthy subjects, always depicting the metahexamide response of the individual patient below his formerly registered tolbutamide-induced changes in serum insulin.

Serum insulinlike activity in 5 elderly diabetics before and after the administration of tolbutamide and metahexamide, respectively. FIGURE 5 gives information as to changes in serum insulinlike activity and blood sugar concentration before and after tolbutamide in 5 elderly diabetics. The fasting serum insulinlike activity levels, already in the range of or slightly above those measured in the normal subjects, again increased in correspondence to the fall in blood sugar within 30 min. after injecting 25 mg. tolbutamide/kg. body weight. These increases amounted to 2600 and to 4300 μU./ml. serum in the third and fourth patients of this group, respectively, the fasting blood samples of which had yielded the highest insulinlike activity levels in serum at the start of the response test as well.

In view of the postulated correlation between increases in serum insulinlike activity and decreases in blood sugar it should be noted that the absolute and the relative rises in insulinlike activity again seemed to be of minor importance. This may be concluded from the comparative responses of the second and fourth patients in this group. In the former, the blood glucose levels decreased

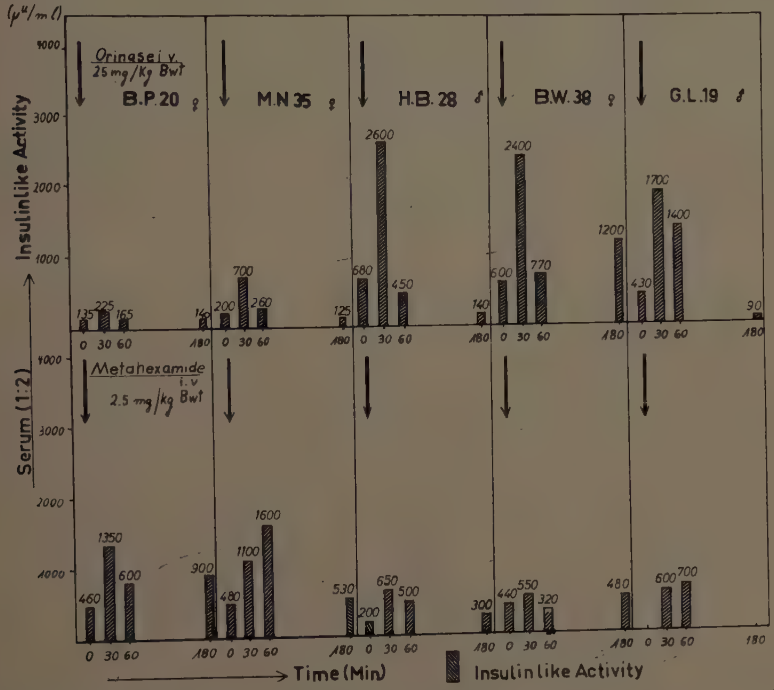

FIGURE 4. Comparative increases in serum insulinlike activity in 5 normal subjects following tolbutamide (Orinase) and metahexamide, respectively.

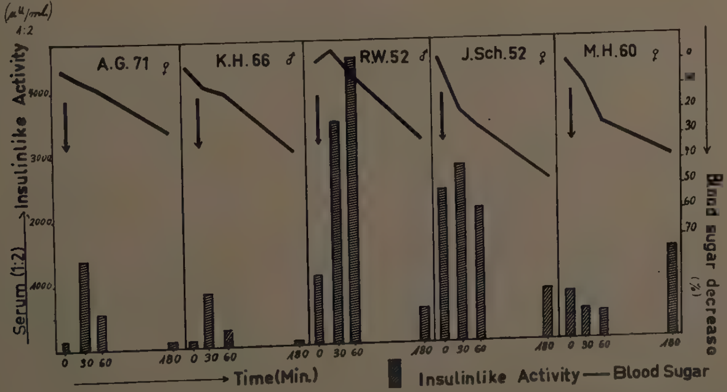

FIGURE 5. The response of elderly diabetics to a single dose of tolbutamide (25 mg./kg., intravenously).

more intensively and rapidly than in all diabetics tested, whereas the insulinlike activity rose only from 470 to 1000 μU./ml. serum; that is, by about 100 per cent. In the latter, a slightly smaller and only slowly progressive decrease in blood glucose resulting in but a primary rise one half hour after the injection of tolbutamide was accompanied by the increase in insulinlike activity from 680 to 4300 μU./ml. (about 600 per cent) within the same interval.

FIGURE 6 shows the results obtained in the 5 diabetics before and after the injection of 2.5 mg. of metahexamide/kg. body weight. They are the same as those observed following the tenfold larger dose of tolbutamide. It may be seen from this figure that 3 hours after the injection of the drug the serum insulinlike activity levels have, in general, returned to the initial ranges, as has been observed in the studies carried out in normal individuals. Contrary to the situation in nondiabetic subjects, however, the hypoglycemia still persisted.

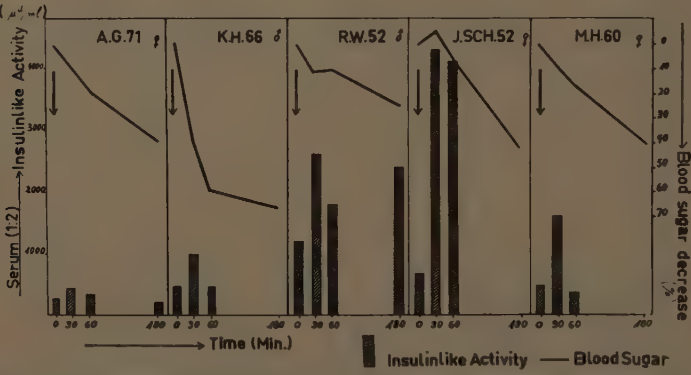

FIGURE 6. The response of elderly diabetics to a single dose of metahexamide (2.5 mg./kg., intravenously).

Something of a consistency in the responses of the individual subjects to the application of both drugs is shown in FIGURE 7. The highest posttolbutamide levels in serum insulinlike activity previously recorded in the third and fourth patients have been determined in the same subjects in the metahexamide studies, as well. It should be mentioned that both patients had never before been under maintenance therapy with sulfonylurea compounds.

Serum insulinlike activity in 4 juvenile diabetics before and after the administration of tolbutamide and metahexamide, respectively. FIGURE 8 demonstrates the blood sugar and serum insulinlike activity levels determined in the 4 juvenile diabetics before and after tolbutamide and metahexamide, respectively. The 4 patients previously were shown to be nonresponsive to sulfonylurea therapy. In accord with this finding, no increases in insulin levels and decreases in blood sugar were observed following the administration of a single dose of the sulfonylureas. The insulin levels nearly in the range of the normals and of elderly diabetics are explained by the comparatively short interval of only 24 hours between the last injection of exogenous insulin and the institution of the

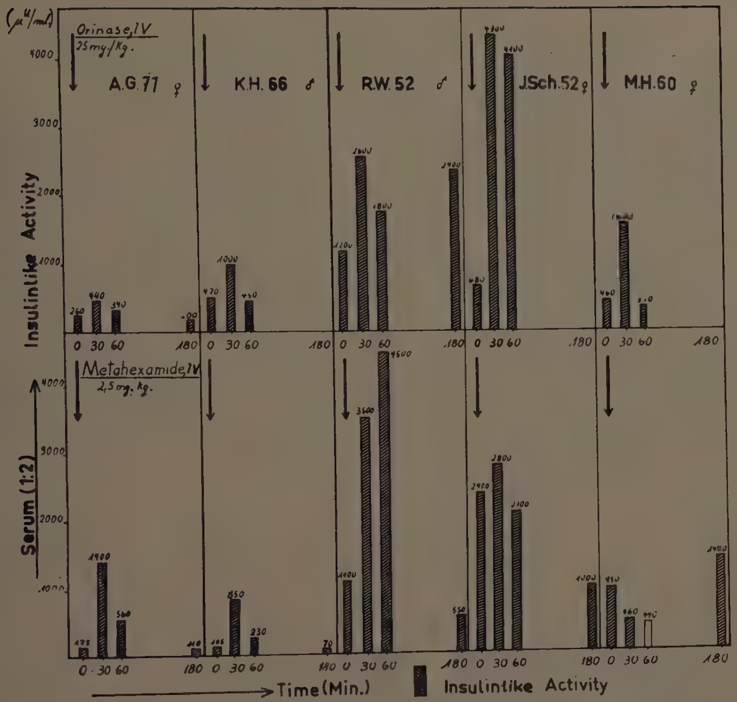

FIGURE 7. Comparative increases in serum insulinlike activity in 5 elderly diabetics following tolbutamide and metahexamide, respectively.

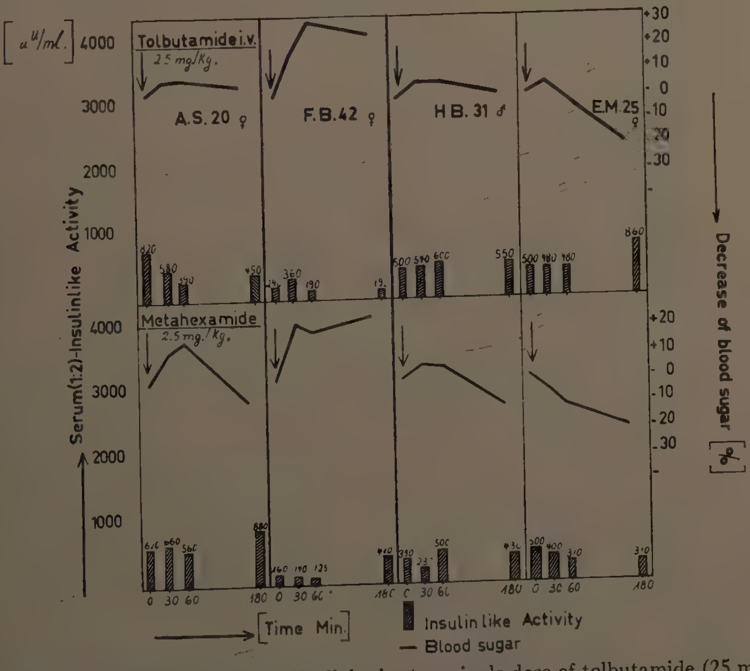

FIGURE 8. The response of juvenile diabetics to a single dose of tolbutamide (25 mg./kg., intravenously) and metahexamide (2.5 mg./kg., intravenously), respectively.

response test. When the insulin administration was discontinued for 48 hours, the serum insulinlike activity was found to be significantly below the values determined in the nondiabetics. However, hyperglycemia and acidosis occurred simultaneously. Since it was felt that comparisons with the other group of diabetics were possible only under the provision of undisturbed metabolism in both, the response tests carried out after an interval of 24 hours were preferred. The comparison of all results (FIGURE 9) shows that the increases in mean serum insulinlike activity levels were most pronounced in the elderly diabetics following the injection of both tolbutamide and metahexamide.

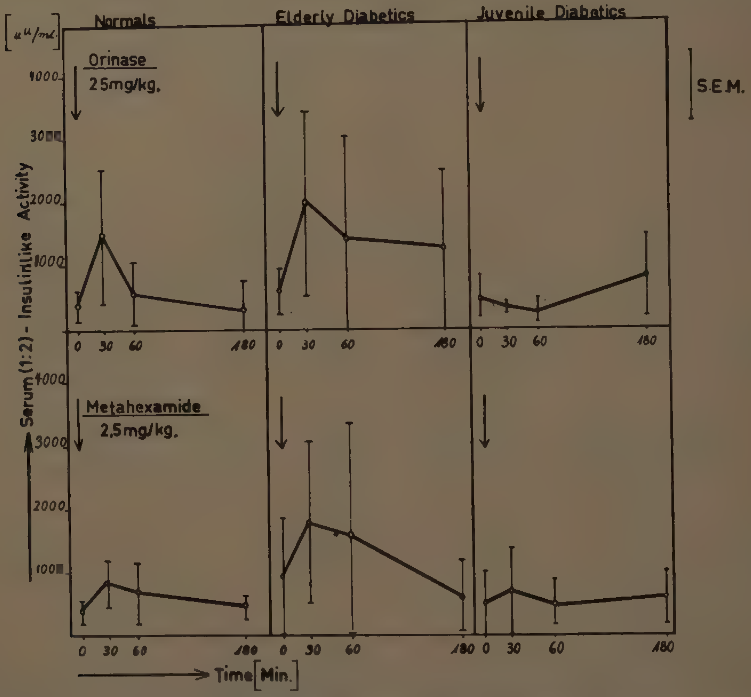

FIGURE 9. Mean of serum insulinlike activity levels in different groups of human subjects before and after tolbutamide (Orinase) and metahexamide, respectively.

In the nondiabetic subjects, on the other hand, the rises in serum insulinlike activity were, in particular following metahexamide, less pronounced. In the elderly diabetics, however, metahexamide has been found to be equivalent in its insulin-increasing capacity to the tenfold higher dose of tolbutamide. It is noteworthy that the rises in serum insulinlike activity following both sulfonylurea compounds occurred in the elderly diabetics as rapidly as in the normals. The juvenile diabetics failed to demonstrate increased insulin activity levels in serum following both tolbutamide and metahexamide.

Serum insulinlike activity associated with an islet-cell adenoma of the pancreas before and after the administration of tolbutamide and metahexamide, respectively. As far as timing of the increases in serum insulin activity and the relation between the dosages and effects of both drugs are concerned, the same results as in normal subjects and elderly diabetic subjects were obtained in a female patient suffering from an islet-cell tumor of the pancreas. TABLE 1 gives information as to the fasting blood sugar and serum insulinlike activity values in this patient, which were determined on 13 occasions. The insulinlike activity levels in serum exceeded the normal range 11 in 13 times. Following tolbutamide and metahexamide intravenously (FIGURE 10), the same rapid and marked elevation of serum insulin occurred within 30 min. after injection, as has been described above in the normals and elderly diabetics. Apparently because of the height of the initial levels, the insulinlike activity increased to more than 6000 μU./ml. serum on one occasion. An extraordinarily large solid insulinoma (FIGURE 11) that weighed 129 gm. and consisted throughout of β-cells was removed from the tail of the pancreas at operation.

TABLE 1

BLOOD SUGAR AND SERUM INSULIN VALUES IN A FEMALE PATIENT WITH AN ISLET-CELL TUMOR OF THE PANCREAS

Blood sugar (mg.%)	Insulinlike activity (μU./ml.)	Date
		1958
48	2100	Nov. 21
45	1700	Nov. 26
85	940	Nov. 28
81	210	Nov. 29
66	4900	Dec. 2
69	1050	Dec. 4
76	1500	Dec. 5
67	2500	Dec. 6
48	2200	Dec. 8
75	170	Dec. 10
63	3800	Dec. 15
		1959
45	2500	Jan. 8
62	800	Jan. 10

Studies in Animals

Materials and Methods. These experiments were designed to study differences between peripheral and portal venous insulin levels before and after the administration of the sulfonylureas and the comparative effects of both tolbutamide and metahexamide upon release of insulin from the pancreas. Inbred Wistar rats weighing 150 to 200 gm. were used throughout the studies and were fed ad libitum until the start of the experiments. They were divided into groups of 4. The femoral and portal veins were cannulated by small plastic tubes. Blood sampling for determinations of serum insulinlike activity and blood sugar was performed at zero time and 30 min. after injection of the sulfonylureas (100 mg. of tolbutamide and 10 mg. of metahexamide/kg. body weight) into one of the tail veins. The animals were sacrificed 30 and 60 min. after injection. Their pancreas were removed immediately after death for histological and histochemical examinations.

Diluted serum (1:5) was used in triplicate for measuring insulinlike activity, as in the studies in humans, according to the method of Martin *et al.*[4] In the cytoplasm of the β-cells the content of SS and SH groups was determined in collaboration with Sandritter and Becker according to the method of Barnett and Seligman.[9, 10] The modification of Bahr[11] was used since it is suitable for evaluating photometrically the staining reactions. As has been shown previously in studies carried out on the pancreas of calves[12] in which the amount of extractable insulin was compared with the cytophotometric absorption figures (Sandritter *et al.*[13]), this method allows us to estimate roughly the intracellular content of insulin and insulin precursors in the β-cells.

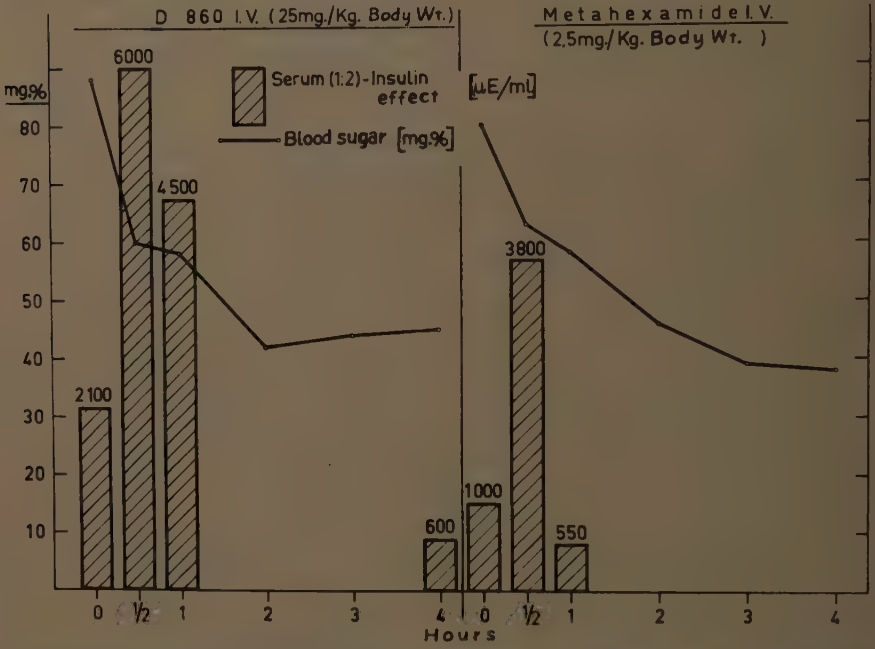

FIGURE 10. Response of a patient with insulinoma to a single dose of tolbutamide and metahexamide, respectively.

Results

FIGURE 12 shows, in a combined fashion, the changes in blood glucose, serum insulinlike activity in portal and peripheral circulation, and the content of SS and SH groups of pancreatic β-cells that were established in the rats before and after administration of tolbutamide and metahexamide, respectively. As in the former experiments in dogs, the normal serum insulin levels were much higher in portal than in peripheral blood in the rats. Thirty minutes after injection of the sulfonylureas, the decreases in blood sugar and in the content of disulfide groups in the pancreas coincided with remarkable increases in serum insulin in the portal venous blood, whereas only a small rise in insulin levels was observed in general circulation.

Thus, the observation made previously in only two dogs,[2,3] that the increase in blood insulin induced by tolbutamide may be found in the circulation mainly proximal to the liver was re-established in the present experiments performed on rats. Moreover, it should be emphasized that this difference between the variations of the insulin levels in portal and general circulation after sulfonyl-

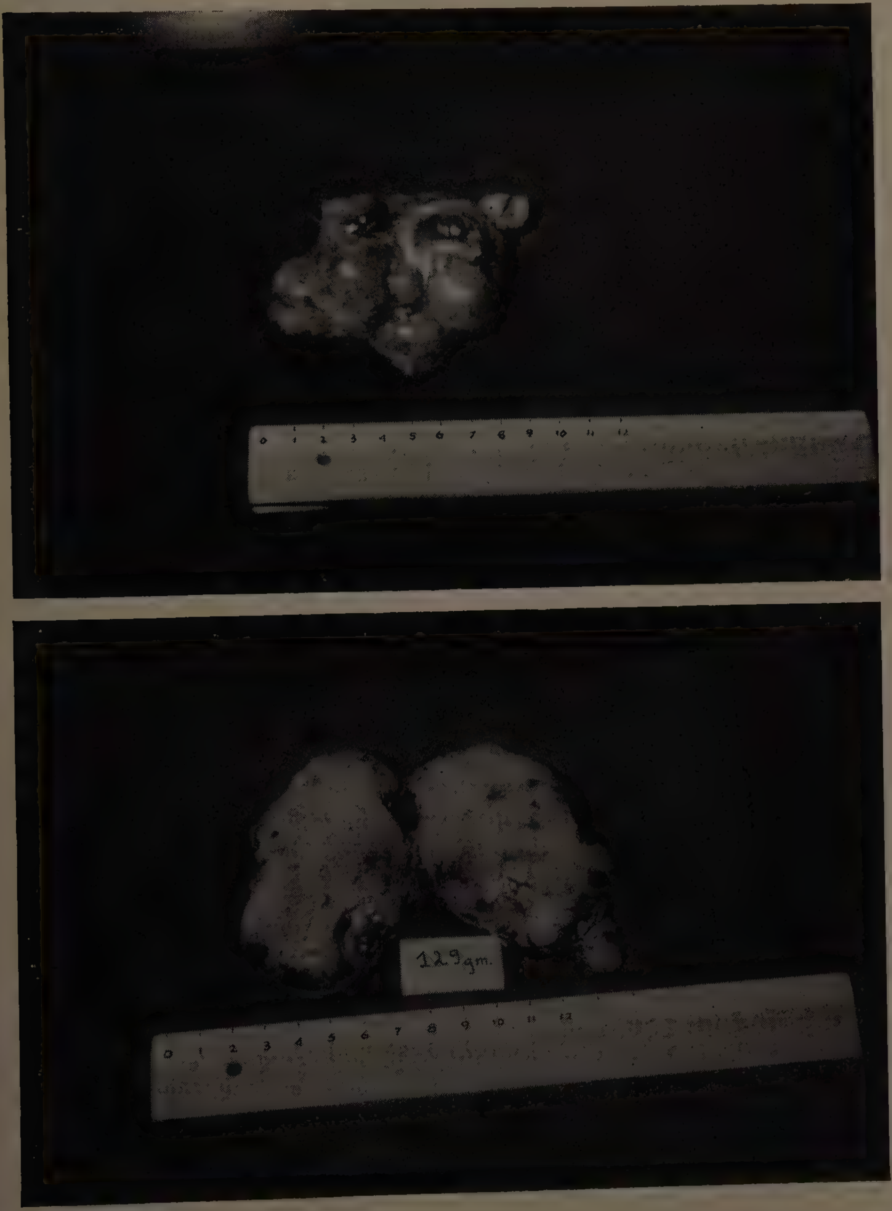

FIGURE 11. Top; an extraordinarily large solid insulinoma (see text). Bottom; tumor opened.

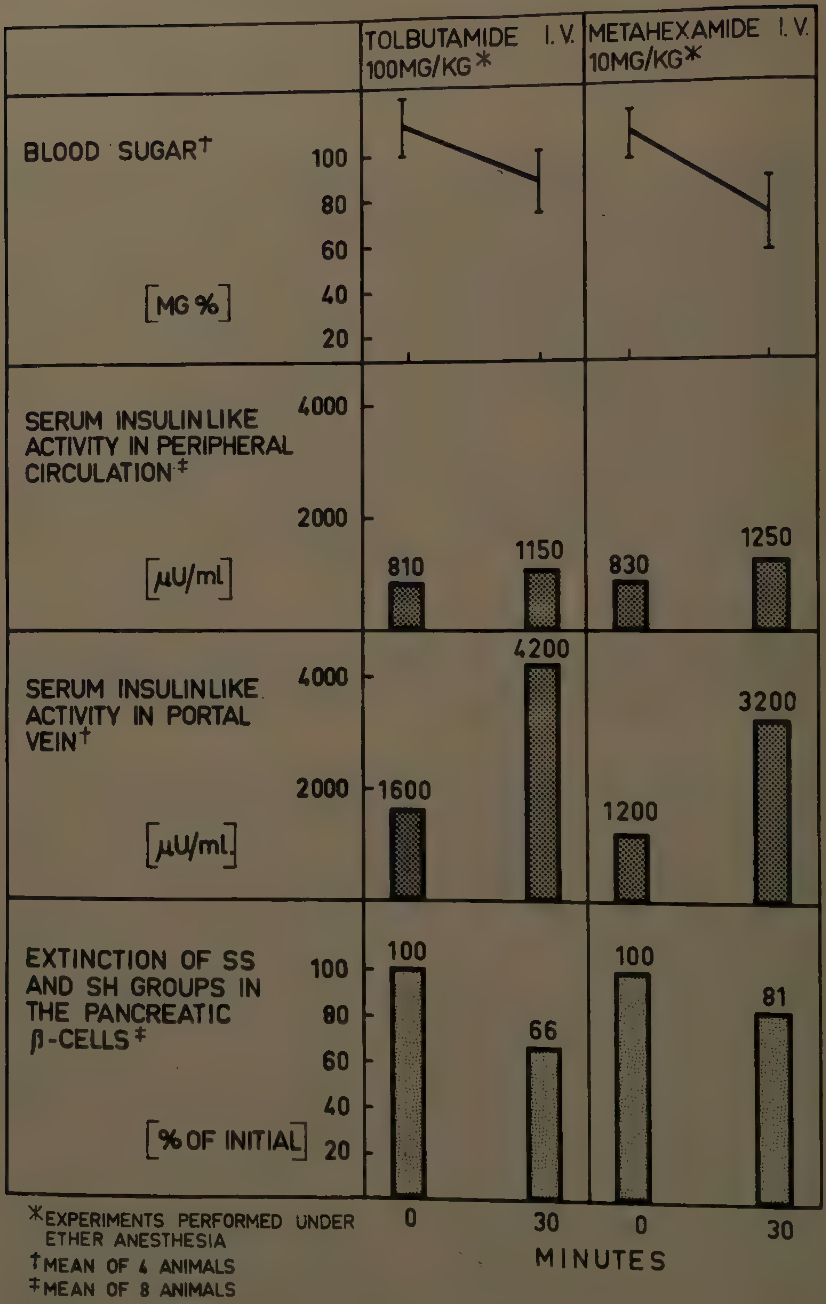

FIGURE 12. Changes in blood glucose, serum insulinlike activity in portal and peripheral circulation, and the content of SS and SH groups of pancreatic β-cells in the rat, determined before and after the administration of tolbutamide and metahexamide, respectively.

ureas is based here upon repeated determinations in a total of 12 rats in the tolbutamide and the metahexamide groups, respectively.

As regards the rather moderate decreases in blood glucose observed at the 30-min. period, it should be understood that ether anesthesia was used throughout the present experiments. In unanesthesized rats the blood sugar underwent an average decrease within the same interval to about 50 per cent of the initial value.

With respect to the comparison between the actions of both drugs on the pancreas, it should be noted that the variations induced by the metahexamide were nearly equivalent to those obtained with the tenfold higher dose of tolbutamide.

Discussion

Our results clearly indicate once again that endogenous insulin is released from the pancreas following the administration of tolbutamide and metahexamide.

As far as the studies in man are concerned this conclusion may be reached from the increase in the levels of serum insulin activity established in the peripheral circulation of nondiabetics of tolbutamide-responsive elderly diabetics, and of one patient with a pancreatic islet-cell tumor 30 min. after the intravenously administered sulfonylureas. Conversely, no elevations of serum insulin activity were found in the juvenile diabetics not suitable for sulfonylurea therapy.

The validity of these results is significantly enhanced by the fact that the same observations regarding rises in insulin levels after tolbutamide in normals and in elderly diabetics recently have been made by Vallance-Owen and his group.[14a,14b] Their experiments were done blind, in 5 normal subjects and 17 diabetics both before and after tolbutamide by mouth, and the rat hemidiaphragm method was used for measuring the plasma insulin activity. Thus, it seems justifiable to stress the fact that, despite contradictory findings in the past, insulin levels are elevated in peripheral circulation after sulfonylureas.

There is considerable evidence to show that this increase in insulin concentration in general circulation is sufficient to explain the decrease in blood sugar after tolbutamide therapy. The correlation between increases in insulin levels and decreases in blood sugar has been good in the elderly diabetics ($p = 0.05$). In the nondiabetics the maximum increase in insulinlike activity corresponded approximately to the times of the maximum decrease in blood glucose concentration. A rough calculation shows that the amount of endogenous insulin released into general circulation after sulfonylureas is in the range of exogenous insulin administered peripherally in a dose of about 0.05 to 0.1 U./kg. body weight.

On the other hand, there remains a large body of data that is difficult to explain on the basis of accelerated glucose utilization in peripheral tissues alone. For instance, this concept cannot explain the discrepancy between insulin levels already returned to normal and glucose concentrations still hypoglycemic that were found at the 3-hour interval in the tolbutamide-responsive diabetics. This prolongation of the hypoglycemia thus should be attributed to an additional mechanism different from that implied in the increased insulin levels in general circulation.

Attention should therefore be directed toward the differences between insulin activity levels in the portal and peripheral venous blood (that is, proximal to and distal to the liver) established both before and after administration of tolbutamide. This difference was observed only occasionally in our earlier experiments in dogs to such an extent that considerable tolbutamide-induced increases in insulin activity in the portal vein contrasted with the failure of increases in general circulation.[3] It was found in a similar way in the present studies carried out in rats. Remarkable rises in insulin activity occurred in the portal venous blood, whereas only a small increase was observed in general circulation. This points, indeed, to the hepatic trapping or binding of endogenous insulin discharged from the pancreas after sulfonylureas. It may be more-or-less quantitatively pronounced in the different species, permitting only small amounts of insulin to pass into general circulation, as occurred in the rats, for example, whereas considerable concentrations of pancreatic insulin initially reach the circulation after the liver in the human beings. If this hypothesis should prove correct, further investigations in men may be profitably directed toward the involvement of the hepatic binding of endogenous insulin in the second phase of the hypoglycemia induced by tolbutamide, which cannot, as shown in the diabetics, be explained by concomitant increases in insulin levels in peripheral circulation. Hence, one may be inclined to believe that only the primary phase of the hypoglycemia after tolbutamide is due to the release of endogenous insulin into peripheral circulation, whereas the prolongation of the hypoglycemia is caused by some other, presumably liver-connected, mechanism.

Although it would be unwarranted to draw very far-reaching conclusions from these studies performed in only a small number of cases, it should be emphasized that the primary increase in serum insulin activity following both sulfonylureas appeared as rapidly and markedly in the elderly diabetics as in the nondiabetic subjects. This finding definitely speaks against the opinion, suggested elsewhere,[15,16] that only minor stores of pancreatic insulin are available for immediate release in the pancreas of elderly diabetics who are suitable for sulfonylurea therapy. The problem of the pathogenesis of diabetes occurring after the age of 40 (maturity-onset type) becomes even more puzzling, however, when we consider this observation. Adding to it the essentially normal or even slightly elevated fasting levels in insulin activity that were found in the elderly diabetics by a number of authors previously[17-20] and again in the studies reported, one may be led to inquire why these patients have been classified as diabetics.

It was the purpose of this paper, furthermore, to compare two sulfonylurea compounds, tolbutamide and metahexamide, with regard to their capacity to stimulate pancreatic insulin secretion. On the basis of the increases in serum insulinlike activity and the decreases in the insulin content of the rat pancreas, metahexamide appears, under the present experimental conditions, to be roughly equivalent to the tenfold larger amount of tolbutamide with respect to stimulation of pancreatic insulin secretion, except in the case of the nondiabetic subjects. No reasonable solution can be offered at this time for the weaker response that has been noticed in normal human subjects. The tenfold greater effect in the elderly diabetics, however, comports with the clinical observation that one tenth of the tolbutamide dose often is sufficient to control the metabolism of patients changed over from tolbutamide to metahexamide.

Two critical points are implied in our studies. The first is represented by the fact, mentioned before by *Martin et al.*,[4] that this bioassay based upon the metabolic acitivity of the rat adipose tissue does not measure insulin activity, but only insulinlike activity. The second point consists of the problem of insulin inhibitors present in each serum. These factors may also disturb the measurements of insulinlike activity in our studies to a considerable extent.

This particular lack of specificity, however, is shared by all bioassays for insulin. As to the insulin-inhibiting substances, it should be re-emphasized that the dilution of the sera in our assays resulted in diminishing the activity of the inhibitors. Eventually, they were involved in all determinations of the present investigation, thus interfering uniformly with all measurements. Hence, it seems reasonable to assume that the differences established between the insulinlike activity levels measured before and after tolbutamide, on the one hand, and in portal and peripheral circulation, on the other, are real differences. Moreover, the elevated fasting levels in insulin activity measured in the patient suffering from a pancreatic β-cell tumor, and the decreased values in serum insulin found in the juvenile diabetics after a prolonged period of discontinuation of exogenous insulin further support this conclusion.

In our opinion, therefore, the fact that the tolbutamide and metahexamide response tests were performed following a single dose of the sulfonylureas deserves more attention. In two elderly diabetics only oral treatment was given beforehand. As it may be inferred from studies previously performed in this laboratory on intact calves[12] in which the content of extractable pancreatic insulin was used as an index for estimating insulin release from the pancreas after tolbutamide, a short period of continuous treatment with these drugs significantly alters the reaction of the normal pancreatic gland to tolbutamide administration. Essentially the same observations have been made by others in normal rats and dogs.[21,22]

Therefore, the reaction to the application of a single dose of tolbutamide by a diabetic patient maintained under oral therapy for an extended period of time must be more thoroughly investigated before our findings can be interpreted correctly.

Summary

Increases in serum insulinlike activity levels have been established in the peripheral circulation of 5 nondiabetics, 5 tolbutamide-responsive diabetics, and of 1 patient with a pancreatic islet cell tumor following treatment with both tolbutamide and metahexamide. No elevation of serum insulin activity was observed in 4 juvenile diabetics who were unsuitable for sulfonylurea therapy after the injection of the 2 compounds.

Although the increases in insulin concentration in general circulation were found to be sufficiently high to explain the blood sugar decreases after sulfonylureas, it is our opinion that only the primary phase of hypoglycemia after sul-

fonylureas is due to the release of endogenous insulin into the peripheral circulation, whereas the prolongation of the hypoglycemia is caused by some other mechanism, presumably liver-connected.

This view is supported by the demonstration of differences between insulin activity levels in portal and peripheral venous blood established both before and after one administration of tolbutamide and metahexamide in intact rats. Remarkable sulfonylurea-induced rises in serum insulin in the portal vein contrasted with only small increases in general circulation. With regard to similar observations made previously in other animal species, the concept of some kind of a hepatic binding of endogenous insulin discharged from the pancreas after tolbutamide is supported by these findings and needs further clarification.

On the basis of the increases in serum insulinlike activity established in the elderly diabetics, the patient with an insulinoma, and the rats, as well as the decreases in the insulin (for example, the disulfide group) content of the rat pancreas, metahexamide appears to be roughly equivalent to the tenfold larger amount of tolbutamide with respect to pancreatic insulin secretion under the present experimental conditions.

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DETERMINATION OF METAHEXAMIDE IN HUMAN PLASMA

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Metahexamide [1-(3-amino-*p*-tolylsulfonyl)-3-cyclohexylurea] is a highly potent, orally active, antidiabetic agent belonging to the class of arylsulfonylureas. Correlation of plasma levels of metahexamide with reduction in blood sugar concentrations requires a suitable analytic procedure.

Several methods have been reported previously for the determination of antidiabetic arylsulfonylureas. Spingler and Kaiser,[1] Forist *et al.*,[2] and Bladh and Nordén[3] have described methods for the determination of tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) based on the intense absorption by this compound at 228 mμ. The procedure of Toolan and Wagner[4] for chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-propylurea] similarly is based on ultraviolet spectroscopy. Visible spectrophotometric methods for tolbutamide determination have been reported by Spingler,[5] by McDonald and Sawinski,[6] and by Chulski.[7] Carbutamide (1-butyl-3-sulfanilylurea) has been determined colorimetrically by diazotization of the aromatic amino group, followed by coupling with thiocol.[8] Metahexamide also contains an aromatic amino group capable of diazotization and coupling. However, in this case the amino group is in the *meta* position relative to the sulfonamide group instead of the *para* position as is true in carbutamide and in the sulfa drugs. Moreover, in metahexamide a methyl group is also present *ortho* to the amino group. Because of these structural differences, the behavior of metahexamide in a modification of the Bratton-Marshall procedure[9] has been critically examined and is reported herein.

Materials and Methods

The four following *reagents* were used: (1) perchloric acid, 10 per cent (a sample of 31.3 ml. of 70 per cent perchloric acid is mixed with 300 ml. of water); (2) sodium nitrite, 0.1 per cent; (3) ammonium sulfamate, 0.5 per cent; and (4) N-(1-naphthyl)-ethylenediamine dihydrochloride (NED), 0.1 per cent (prepared fresh daily).

Apparatus. A Beckman Model B spectrophotometer equipped with 1-cm. cells was employed for absorbance measurements.

Procedure. One ml. of plasma (or serum) is mixed with 3 ml. of water, and 4 ml. of 10 per cent perchloric acid is added slowly, with gentle agitation. The mixture is swirled occasionally during 15 min. and then filtered through Whatman No. 40 filter paper (a 2-in. funnel with 9.0-cm. paper permits recovery of sufficient filtrate for the next step).

Five ml. of the proteinfree filtrate is added to a 25-ml. Erlenmeyer flask. One ml. of 0.1 per cent sodium nitrite is added and the solution thoroughly mixed. Diazotization is allowed to proceed for 10 min., after which the excess nitrite is destroyed by the addition of 1 ml. of 0.5 per cent ammonium sulfamate. The solution is thoroughly mixed and, after 3 min., 1 ml. of 0.1 per cent NED is added. The resulting solution is mixed by swirling several times and then rapidly transferred to the spectrophotometer cell, which should be filled as completely as possible. Absorbance of this solution is determined at 535 mμ versus a reagent blank between 5 and 30 min. after the addition of the NED to the sample. A plasma blank (containing no metahexamide) is carried through the same procedure.

The plasma metahexamide concentration (mg./100 ml.) is calculated from the equation:

$$M = (A_S - A_B)/\mathrm{a}_{535}$$

where

A_S = observed absorbance for the sample at 535 mμ

A_B = observed absorbance for the plasma blank at 535 mμ

a_{535} = absorbance per milligram of metahexamide per 100 ml. of plasma for metahexamide added to plasma and carried through the standard procedure (about 0.097).

TABLE 1

DIAZOTIZATION OF METAHEXAMIDE AT 25° C.

Time (min.)	a_{535}	
	Pseudofiltrate	Plasma filtrate
1	0.070	—
3	0.101	—
5	0.105	—
10	0.105	0.0962
20	0.104	0.0958
30	—	0.0960
40	0.102	—
60	0.100	—

Results and Discussion

The procedure presented is a modification of the method of Bratton and Marshall[9] for the determination of aromatic amines involving diazotization followed by coupling of the diazonium salt with NED. Metahexamide yields an azo compound with an absorption maximum at 535 mμ.

In order to define completely the limitations and allowable variations in the application of the method to metahexamide, a careful examination of experimental variables has been made. This has been accomplished by the use of pseudofiltrates (5 per cent perchloric acid) containing metahexamide and, in some cases, with plasma filtrates obtained from human plasma containing added metahexamide.

Because the amino group in metahexamide is *ortho* to a methyl group and *meta* to the sulfonamide grouping, rather than *para*, as is the case in carbutamide and in the sulfa drugs, the rate of diazotization has been studied. TABLE 1 shows that 5 min. are required for maximal reaction, and that the diazonium salt is stable in the reaction mixture (either pseudofiltrate or plasma filtrate) for at least an additional 25 min. A 10-min. reaction period has been adopted for routine use. It can be seen that the response obtained with metahexamide in the plasma filtrate is highly reproducible and is about 91 per cent of the response obtained with standards in the pseudofiltrate.

To avoid reaction with the coupling reagent (NED), excess nitrite is destroyed by the addition of ammonium sulfamate. To define allowable variations in time at this step, stability of the diazonium salt in the reaction mixture containing excess ammonium sulfamate has been examined. TABLE 2 shows that destruction of nitrite is complete within 1 min., and that the diazonium salt is stable for at least 1 hour. A 3- to 5-min. reaction period has been employed for routine use, but considerable flexibility is possible.

TABLE 2

STABILITY OF DIAZOTIZED METAHEXAMIDE IN THE PRESENCE OF EXCESS AMMONIUM SULFAMATE AT 25° C.

Age of solution (min.)	a_{535}*
1	0.105
3	0.105
5	0.104
10	0.105
20	0.104
40	0.103
60	0.104

* Five per cent $HClO_4$ pseudofiltrate.

TABLE 3

STABILITY OF THE AZO COMPOUND FROM METAHEXAMIDE

Time after NED addition (min.)	a_{535}*		
	Pseudofiltrate	Plasma filtrate	TCA pseudofiltrate
3	0.102	0.0939	0.0940
5	0.104	0.0958	0.0937
7	0.104	0.0960	—
10	0.104	0.0962	0.0925
15	0.104	0.0962	0.0913
20	0.104	0.0964	—
25	0.103	0.0964	—
30	0.103	0.0964	—
45	0.103	0.0966	0.0893
60	0.101	0.0964	0.0893

* Sample held in 1-cm. spectrophotometer cell.

Rate of formation and stability of the azo compound following addition of the NED has been determined. TABLE 3 shows that 5 min. are required for maximum color formation in both the pseudofiltrate and the plasma filtrate. Some loss occurs in the pseudofiltrate after about 30 min. in the spectrophotometer cell. Color stability is somewhat greater for the plasma filtrate, with no loss occurring over a period of an hour. However, solutions left in the reaction vessel (a 25-ml Erlenmeyer flask) show considerable run down in absorbance. This has been traced to an oxidative process. For this reason it is recommended that the solution be transferred to the spectrophotometer cell rapidly following addition of the NED to the sample. This procedure produces a situation where the surface-to-volume ratio is low and therefore less favorable to air oxidation.

Included in TABLE 3 are the results obtained when a sample of metahexamide in a trichloroacetic acid pseudofiltrate, prepared according to Bratton and Marshall,[9] is examined for color production and stability. The response is about 90 per cent of that obtained with the perchloric acid system, and the color is considerably less stable. For these reasons, perchloric acid has been employed as protein precipitant.

TABLE 4

EFFECT OF AGING OF THE PROTEIN PRECIPITATE ON RECOVERY OF METAHEXAMIDE FROM HUMAN PLASMA

Age of Suspension (min.)	a_{535}
15	0.0954
30	0.0966
45	0.0973
60	0.0962

TABLE 5

STABILITY OF METAHEXAMIDE IN A 5 PER CENT PERCHLORIC ACID PSEUDOFILTRATE AT 25° C.

Age of solution	a_{535}
5 min.	0.104
1 hr.	0.105
2	0.105
9	0.105
25	0.105

The effect on the recovery of metahexamide of the exposure of precipitated plasma proteins to perchloric acid has been studied. TABLE 4 shows that recovery is constant over a period of an hour. Stability of metahexamide in a pseudofiltrate is shown in TABLE 5. No loss is observed over a period of 25 hours, indicating that the proteinfree filtrates need not be analyzed immediately after collection.

Beer's law is followed for metahexamide concentrations of 0 to 8 mg./100 ml. However, as indicated above, the slope of the calibration curve obtained when metahexamide is added to human plasma and carried through the complete procedure is about 91 per cent of that obtained with standards in the pseudofiltrate, probably reflecting losses due to adsorption on the protein precipitate. For this reason, calculations should be based on the response observed for metahexamide added to human plasma, as outlined in the procedure above.

Analysis of plasmas containing no metahexamide (plasma blanks) has indicated an apparent metahexamide concentration of 0.1 to 0.2 mg./100 ml. Therefore, for maximum accuracy, especially at low levels, a plasma blank should be run and the appropriate correction applied as described above.

Application of the method to the determination of metahexamide added to human plasma has given the results shown in TABLE 6. The mean recovery plus or minus standard deviation of 100.4 ± 1.9 per cent indicates excellent accuracy and precision.

TABLE 6
RECOVERY OF METAHEXAMIDE ADDED TO HUMAN PLASMA

Added (mg./100 ml.)	Found (mg./100 ml.)	Recovery (%)
	Plasma 1	
0.94	0.95 0.96	101.1 102.1
1.89	1.95 1.96	103.2 103.7
2.83	2.73 2.82	96.5 99.6
3.78	3.71 3.78	98.1 100.0
4.73	4.64	98.1
	Plasma 2	
0.94	0.93	98.9
1.89	1.94 1.89	102.6 100.0
2.83	2.87 2.84	101.4 100.4
3.78	3.82 3.81	101.1 100.8
4.73	4.69 4.71	99.2 99.6

Mean ± standard deviation 100.4 ± 1.9%.

The procedure as presented is useful over the range 0.2 to 8 mg. metahexamide/100 ml. plasma. Greater sensitivity, if needed, should be possible by employing a larger plasma sample while maintaining the same volume of proteinfree filtrate, provided, that suitable calibration is done. The method is rapid, accurate, and precise. The plasma blank encountered is small, and only other aromatic amino compounds interfere. Direct extension of the method to the analysis of urine is possible, recognizing that metabolites as well as intact metahexamide are determined.

Summary

A modification of the Bratton-Marshall[9] procedure for the determination of aromatic amines has been developed for the determination of metahexamide in human plasma. Greater color stability and somewhat higher sensitivity have resulted from the substitution of perchloric acid for trichloroacetic acid as the protein precipitant. Experimental variables have been thoroughly evaluated. Under the conditions described, recovery of added metahexamide from human plasma over the range 1 to 5 mg./100 ml. has been 100.4 ± 1.9 per cent (mean ± standard deviation).

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DETERMINATION OF THE HALF LIFE OF METAHEXAMIDE IN NORMAL HUMANS

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Metahexamide [1-(3-amino-*p*-tolylsulfonyl)-3-cyclohexylurea] is a new experimental antidiabetic sulfonylurea. Before extensive clinical evaluation of this new compound on diabetics, the time for halving blood levels of metahexamide (that is, the effective half life) was determined. This paper summarizes the clinical investigation (L.L.M.) and the data analysis (E.R.G.).

Procedure

Twelve normal nonfasting subjects were used; 9 were males and 3 were females. All cases but patient Wa. were white; all but one were ambulatory volunteers who carried on their normal daily activities except for the times at which specimens were taken. The exception was patient Wa., hospitalized with bilateral tibial fractures and, at that time, at bed rest. The subjects were all seen at 9:00 A.M., when blood for the zero-hour specimen was taken and 200 mg. of metahexamide was administered with a glass of water. Following this, blood specimens were taken at intervals of ½, 1, 2, 6, 12, 24, 48, 72, and 96 hours. Plasma specimens were analyzed for the metahexamide blood level by the method described by Forist, elsewhere in this monogram. This method is a modification of the Bratton-Marshall procedure;[1] the nonfasting blood sugar level was determined by the method of Folin and Wu.

Results and Discussion

Typical plots of the log metahexamide blood concentration versus time in hours are shown in FIGURE 1. The rates of elimination from the blood are summarized in TABLE 2 as based on the data in TABLE 1 corrected for the zero-hour value. Included in TABLE 2 are the slopes of the semilogarithmic plots; the derived rate constants, k; the half lives, $t_{1/2}$, of metahexamide elimination from the blood; the number of points serving as a basis for a rate estimate; the hour of maximum value attainment from the 1-, 2-, and 6-hour values; and the maximum value in milligrams per cent.

The basic equation for first-order elimination from the blood is:

$$\log A = -(k/2.303)t + \log A_0 \qquad (1)$$

where A_0 is the amount of drug at zero time (extrapolated) and the first-order rate constant, k, is in units of reciprocal time, that is, hour^{-1}. The slope, $S = k/2.303$, can be transformed to the half life $t_{1/2}$, the time at which half the drug is eliminated, by the expression:

$$\frac{-2.303 \log 0.5}{k} = \frac{6.91}{k} = \frac{2.30}{S} = t_{1/2} \qquad (2)$$

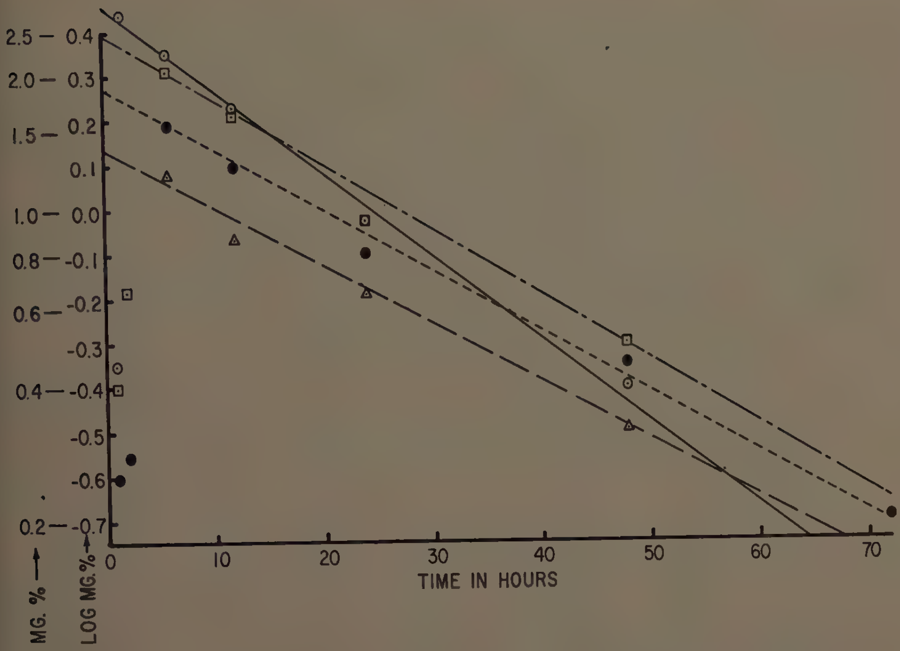

FIGURE 1. Typical plots of log metahexamide in milligrams per cent versus time in hours for individual patients on 200 mg., orally. Symbols: ⊙, patient S'h.; △, patient Br,; ⊡, patient Ja.; and ●, patient Pe.

TABLE 1

PLASMA METAHEXAMIDE AND BLOOD SUGAR

(Plasma Metahexamide in Milligrams Per Cent (First Row)[a] and Blood Sugar in Milligrams Per Cent (Second Row)[b] on Administration of 200 mg. Metahexamide Single Dose Orally; Normal Subjects, Nonfasting; Blood Sugars, Nonfasting)

Name	0	½	1	2	6	12	24	48	72	96
Du.	0.1[a] 108[b]	1.3 88	1.4 80	1.7 86	1.35 113	1.3 108	1.05 90	0.43 106	0.23 97	0.12 100
Wa.	0.05[a] 92[b]	0.05 88	0.1 96	0.2 80	1.25 92	85 100	0.25 94	0.15 108	0.12 95	0.10 93
S'h.	0.1[a] 113[b]	0.25 103	0.55 100	2.82 94	2.35 98	1.80 103	1.05 100	0.50 113	0.22 98	
Du.	0.1[a] 110[b]	0.45 170	0.98 109	.80 103	0.65 98	1.05 92	0.62 100	0.35 94	0.1 113	0.05 103
Mu.	0.2[a] 108[b]	0.4 110	0.9 105	2.0 119	1.75 103	1.1 119	0.80 103	0.15 125		0.1 122
Ja.	0.15[a] 114[b]	0.3 92	0.55 95	0.80 110	2.2 106	1.8 116	1.1 103	0.65 110		0.21 110
Br.	0.05[a] 124[b]	0.1 119	0.1 108	0.15 98	1.25 106	0.91 103	0.70 100	0.37 116		0.08
Do.	0.05[a] 122[b]	0.08 129	0.1 110	0.55 106	1.10 106	0.70 98	0.37 113	0.25 103		0.05
Si.	0.08[a] 98[b]	0.09 98	0.10 90	0.15 103	1.12 96		0.65 103	0.15	0.08	0.05
Op.	0.07[a] 103[b]	0.10 86	0.15 92	0.22 98	1.30 98		0.88 96	0.10	0.07	0.03
Pe.	0.10[a] 125[b]	0.25 153	0.35 149	0.38 100	1.65 92	1.35 88	0.90 95	0.55 93	0.30 103	0.15
Ha.	0.15[a] 88[b]	0.30 120	1.22 94	1.70 82	1.15 103	1.00 91	0.72 85	0.20 93	0.15 98	0.10

It is our opinion that, although a rate constant or half life derived from the pooling of individual's data obtained at the same time after administration may serve as a value useful in comparison with another drug, yet it has little significance in estimating human variability in maintenance of drug blood levels. A better choice of data treatment is to determine half-life values of the individuals.

Such data are given in TABLE 2. The mean half life is 18.2 hours, the standard deviation σ of an individual being 5.7 hours. The estimated tolerance limits[2] within which 95 per cent of the population of half lives will fall is 18.2 ± 13.1

TABLE 2

SUMMARY OF RATES OF METAHEXAMIDE BLOOD LEVEL LOSS IN PATIENTS*

Patient	Slope of log blood level versus time	Estimated rate constant for elimination from blood k (hr. − 1)	Half life inblood ($t_{1/2}$ in hours)	No. of points serving as basis of rate estimate**	Hr. of max. value from 1-, 2-, 6-hr. points	Max. value; mg. per cent
Du.	0.0158	0.0564	19.0	6†	2	1.60
Wa.	0.0478	0.110	6.3	3††	6	1.20
S'h.	0.0188	0.0433	15.9	6‡	2	2.72
Du.	0.0153	0.0352	19.6	3	?‡‡	0.95
Mu.	0.0220	0.0507	13.6	4§	2	1.80
Ja.	0.0146	0.0337	20.6	4‡	6	2.05
Br.	0.0132	0.0304	22.6	4‡	6	1.20
Do.	0.0241	0.0556	12.4	3§	6	1.05
Si.	0.0145	0.0534	20.7	2§§	6	1.04
Op.	0.0106	0.0245	28.3	2§§	6	1.23
Pe.	0.0137	0.0316	21.9	5‡	6	1.55
Ha.	0.0175	0.0404	17.1	4§	2	1.55

* The raw data have been corrected for background by subtraction of the zero-hour value. This eliminated any significance to most of the 72- and all of the 96-hour blood levels.

** The estimates of elimination rates were based on the maximum value and subsequent values that were significant. The analytical method given by Forist elsewhere in this monograph gives an absorbance on only ⅒ mg. per cent. Obviously, values of 0.20 mg. per cent or less should be looked on with great skepticism, especially when correction for background is made on only 1 zero value and when background ranges from 0.01 to 0.02 in absorbance, that is, 0.1 or 0.2 mg. per cent.

† Data not particularly consistent internally.

†† In doubt, as one of these points at 0.2 mg. per cent. Also only patient not assured healthy, namely, the Negro with tibial fractures.

‡ Good first-order fit.

‡‡ The plot went through several maxima, obviously indicative of experimental aberration.

§ Fair first-order fit.

§§ Quality of fit unknown, 2 points can only estimate 1 straight line.

TABLE 2 shows that the maximum blood level value occurs in less than 6 hours, but in greater than 2 hours, and ranges from 0.95 mg. per cent to 2.72 mg. per cent for an oral dose of 200 mg., with a median value of 1.35 mg. per cent.

An extreme value in TABLE 2 is that of patient Wa., the only possible exception to the criterion of "healthy" adults, a Negro hospitalized with bilateral tibial fracture, but no other pathology.

The other method for treatment of the data is shown in TABLE 3 and plotted in FIGURE 2. This treats the blood level data, after correction for background by subtraction of the "zero" value, as the "mean" value for all patients at the time of oral administration. The vertical lines in FIGURE 2 represent the standard deviation of the mean.

The large standard deviations at the 0.5-, 1-, and 2-hour values are due to

TABLE 3

STATISTICS OF METAHEXAMIDE BLOOD LEVELS*

(Based on the Mean Value of All 12 Nonfasting Patients at a Given Time After Oral Administration of 200 mg.)

Time in hours	Mean mg. per cent $\bar{X}$	Standard deviation σ	Standard deviation of mean† $\sigma\sqrt{n}$
0.5	0.206	0.329	0.095
1	0.442	0.447	0.129
2	0.856	0.859	0.248
6	1.33	0.47	0.14
12	1.08	0.36	0.12†
24	0.658	0.245	0.071
48	0.225	0.175	0.051

* The raw data have been corrected for background by subtraction of the zero-hour value. This eliminated any significance to the 72- and 96-hour levels.

† So that $n = 12$, $D.F. = 11$ for all hours except the 12-hour values when 2 blood levels were not taken and $n = 10$, $D.F. = 9$.

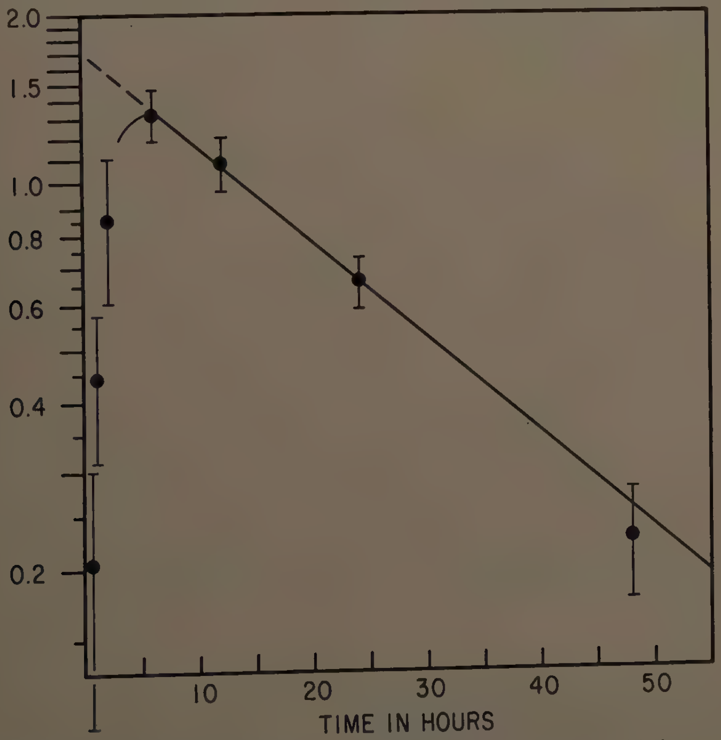

FIGURE 2. Semilogarithmic plot of average of 12 patients; metahexamide in mg. per cent in blood versus time in hours on 200 mg., orally. The vertical lines represent the standard deviations of the mean; $t_{1/2} = 17.3$ hours.

the different absorption rates among the individuals (see TABLE 1). Absorption has been virtually completed by 6 hours; actually, only 3 points (6, 12, and 24 hours) are valid for the estimation of the half life of metahexamide in the plasma. The 48-hour value is significantly greater than zero, but it must be realized that an absorbance of 0.020 indicates an 0.2 mg. per cent of metahexamide, since Forist's method was designed for the range 0 to 5 mg. per cent of metahexamide in the blood.

Inspection of the clinical data at the "zero" hour clearly shows an "apparent" metahexamide concentration of 0.1 to 0.2 mg. per cent in plasma containing no metahexamide. This blank value is consistent with Forist's estimate.

An important item to remember is that any aromatic amine will provide a positive assay artifact, since it will react in the Bratton-Marshall test.[1] For example, the oral administration of phenacetin (acetophenetidine), C_2H_5O-C_6H_5-$NHCOCH_3$, a component of phenacetin, aspirin, and caffeine (PAC), tablets would interfere and give erroneous positive values for metahexamide.

The apparent maximum blood level is achieved well after 2 hours and before 6 hours, and indicates that absorption is not complete in the stomach or that some factor other than gastric absorption is rate-determining in the appearance of metahexamide in the blood. At a gastric *p*H of 2 or 3, the solubility of metahexamide is about 20 to 10 mg./100 cc. At a 200-mg. dose, the total water content of the stomach would have to be 1 to 2 l. for the material to be totally dissolved, on the assumption of instantaneous dissolution.

It thus follows that complete dissolution will probably not occur until the *p*H 6 or 7 of the intestine is reached, where the solubility is 60 to 500 mg./100 cc. In fact, in the passage from the stomach to the intestine, a *p*H of minimum solubility, namely, $pH = 3.7$, will be encountered, that is, 7.2 mg./100 cc.

The higher the stomach acidity, the greater the dissolution, but the greater the protonation of the *ortho* amine ($pKa = 2.4$) of metahexamide. In general, charged compounds are absorbed with difficulty by the gastrointestinal mucosa. The point of minimum solubility (probably in the duodenum) is the *p*H of maximum concentration of noncharged molecules and the point of maximum absorption. As the intestinal *p*H is approached, solubilization by salt formation of the sulfonamide grouping occurs ($pKa = 5.2$).

It is thus readily apparent that metahexamide has a built-in "depot" effect, that is, a relatively prolonged absorption time attributable to the unique interaction of salt formation, solubility, and *p*H variation in the gastrointestinal tract. Of course, an analogous argument can be developed for tolbutamide.

The estimated half life of tolbutamide in human blood is 4 hours,[3] whereas that of metahexamide at 200 mg. oral dose is 16 to 20 hours. Thus it may be concluded that metahexamide has 4 to 5 times the duration of tolbutamide in the blood on oral administration. The half time of chlorpropamide is approximately 32 hours;[4] thus the half life of metahexamide is about one half that of chlorpropamide.

Summary

Pseudo first-order rate plots of metahexamide elimination from human blood on 12 nondiabetic, nonfasting, adult humans given 200 mg. orally showed an

average half life of 18.2 hours, with the standard deviation among individuals of plus or minus (±) 5.7 hours and with a standard deviation of the mean of 1.6 hours. It can be concluded that metahexamide has, within biological variations, 4 to 5 times the half life of tolbutamide in the blood and approximately one half the half life of chlorpropamide.

Acknowledgment

Gratitude is expressed to Homer Kesten and George Mackewen, of the laboratory of the White Plains Hospital for the blood level determinations.

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Part IV. The Status of Oral Agents in the Therapy of Diabetes

PHARMACOLOGICAL STUDIES OF THE SULFONYLUREAS

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A good basis for further development of oral antidiabetics is found in toxicological experiences gained with the preparations tolbutamide and carbutamide, already on the market. I am reporting results of long-term experiments with high dosages of tolbutamide. These results were obtained in cooperation with Scholz *et al.*[1-3] Four years of clinical experiences have proved the accuracy of our previous toxicological prognosis based on results of studies on animals, namely, the absence of toxicity in these preparations. Rules regarding duration, intensity, and variation of toxicological tests with animals have been postulated recently also by Lehman.[4] These rules must be recognized, as they are based on experience. These criteria should be applied when new oral antidiabetics are to be developed.

After the introduction of insulin, death in the majority of diabetics is not the direct consequence of the disturbed metabolism, but is the result of the late complications, particularly of the vascular system. In addition to possessing therapeutic effectiveness, modern antidiabetics should not augment complications or provoke new ones. A prognosis regarding possible side effects can be made only when the drug in question has been administered over a prolonged period. Bearing in mind this consideration, I carried out long-term experiments in rats and dogs with tolbutamide since 1955; in the beginning I worked with very high dosages in order to study the picture of toxicity and the type of possible histopathological damage. Trials with lower dosages must demonstrate whether the same injury, even milder in degree, is provoked by prolonged use, and whether a limited dose may be established that results in no pathological changes. This limit dose should be far higher than the therapeutic dose because very strict measures must be applied in drugs intended for administration over a period of decades as compared to those designed for short-term treatment.

The therapeutic dose of tolbutamide for human beings is approximately 25 mg./kg. when given orally. Therefore I have given rats 250, 500, 1000, and even 2000 mg./kg. orally over a period of 9 months. Mix-bred animals were used in air-conditioned rooms and received a standard diet. The drug was fed only by stomach tube. Dogs received 100 mg./kg. orally over a period of 9 months. Higher dosages cannot be given to dogs for this length of time, since the animals would die of acute hypoglycemia. The results are shown in TABLES 1, 2, and 3. We have done considerably more studies in general pharmacology, but the results are of minor importance in the present context.

For us these studies are the standard against which all other hypoglycemic drugs are to be tested. In Hoechst, Germany, more than 1000 hypoglycemic substances have been synthesized thus far. A few of these substances are more potent than tolbutamide, but up to now not one was found to be as virtually free of toxicity as is tolbutamide. Several years ago I investigated the halogen derivatives of sulfonylureas: *p*-chloropropyl- and *p*-chlorobutyl-sulfonylurea, respectively, are more potent than tolbutamide but at the same time considerably more toxic (TABLES 4, 5, and 6). The higher potency has no favorable relation to the increased toxicity. According to my experience the same is true for all sulfonylureas with a halogen substituted on the benzene ring.

TABLE 1
TOLBUTAMIDE IN RATS, 9 MONTHS, $n = 5$

Dose	(mg./kg., orally)			
	250	500	1000	2000
Liver	no pathological findings	no pathological findings	no pathological findings	slight fatty liver
Kidney	no pathological findings	no pathological findings	no pathological findings	no pathological findings
Heart	no pathological findings	no pathological findings	no pathological findings	no pathological findings
Other organs	no pathological findings	no pathological findings	no pathological findings	hyperplasia of the thyroid

TABLE 2
TOLBUTAMIDE IN RATS, 9 MONTHS, $n = 5$

Dose	(mg./kg., orally)				Control
	250	500	1000	2000	
Hemoglobin	normal	normal	normal	normal	—
Erythrocytes	normal	normal	normal	normal	—
Leukocytes	normal	normal	normal	normal	—
Diff. count	normal	normal	normal	normal	—
Urine					
Sugar	←——without pathological findings——→				—
Protein	←——without pathological findings——→				—
Sediment	←——without pathological findings——→				—
Bile	←——without pathological findings——→				—
Liver glycogen (percentages)	4.3	2.9	2.7	2.5	3.3
Glucose tolerance	—	—	—	nondiabetic	—

TABLE 3
TOLBUTAMIDE IN DOGS, 9 MONTHS, $n = 5$

Dose	(mg./kg., orally) 100
Liver	without pathological findings
Kidney	without pathological findings
Heart	without pathological findings
Blood	Hb, ery., leuko., diff., count: normal
Urine	sugar, protein, bile: negative; sediment: normal
Glucose tolerance	nondiabetic

Another product is metahexamide. This compound was developed in a joint research program together with C. F. Boehringer, Mannheim, Germany. In human beings the potency of this drug is as much as 10 times that of tolbutamide; the therapeutic maintenance dose is approximately 2.5 mg./kg. In

TABLE 4
CHLORPROPAMIDE IN RATS, $n = 5$

Dose	(mg./kg., orally)		
	250	500	2000
General tolerance	duration 4 weeks; 0/5 died	duration 4 weeks; 3/5 died	after 2 days 5/5 died
Liver	fatty + degenerative proc.	fatty ++ degenerative proc.	no histology because of decay
Kidney	1/5 nephrosis	fatty tubuli	
Heart	without pathological findings		

TABLE 5
CHLORPROPAMIDE IN RATS, $n = 5$

Dose	(mg./kg., orally)		
	50	100	Control
Hemoglobin	normal	normal	—
Erythrocytes	normal	normal	—
Leukocytes	normal	normal	—
Diff. count	normal	normal	—
Urine			
Sugar	without pathological findings		—
Protein	without pathological findings		—
Sediment	without pathological findings		—
Bile	without pathological findings		—
Liver glycogen (percentages)	4.2	3.4	4.8
Glucose tolerance	diabetic	diabetic	nondiabetic

TABLE 6
CHLORPROPAMIDE IN DOGS, $n = 3$

Dose	(mg./kg., orally)	
	10	50
General tolerance	2/3 died	2/3 died
Liver	fatty +	fatty ++
Kidney	nephrosis	nephrosis
Heart	fatty +	fatty +++
Blood	normal	normal
Urine	without pathological findings	
Glucose tolerance	diabetic	daibetic

this case, also, increased potency is connected with decreased chronic tolerability despite the fact that the acute toxicity of metahexamide is within the range of tolbutamide. The results obtained from my standard tests are shown in TABLES 7, 8, and 9.

TABLE 7
METAHEXAMIDE IN RATS, $n = 10$

Dose	(mg./kg., orally)				
	100	250	500	1000	2000
General tolerance	after 2½ months, 1/10 died; after 3 months, 5/10 sacrificed	after 2 weeks, 2/10 sacrificed	within 1 to 2 weeks, 10/10 died; resp. killed before spontaneous death	within 7 to 10 days, 5/5 died; resp. killed before spontaneous death	after 2 to 5 days, 5/5 died; resp. killed before spontaneous death
Liver	fatty degeneration ++, necrosis +	fatty degeneration +++, necrosis +	fatty degeneration ++++, necrosis ++	fatty degeneration ++++, necrosis ++	fatty degeneration +++, necrosis
Kidney	fatty + nephrosis	fatty + nephrosis	fatty ++ nephrosis	nephrosis	nephrosis
Heart	without pathological findings	1/10 diff. fatty infiltration +	4/5 diff. fatty infiltration +	2/5 diff. fatty infiltration ++	without pathological findings

TABLE 8
METAHEXAMIDE IN RATS

Dose	(mg./kg.) 100	Control
Hemoglobin	normal	—
Erythrocytes	normal	—
Leukocytes	9100	12200
Diff. count	normal	—
Urine		
Sugar	negative	negative
Protein	+	±
Sediment	negative	negative
Liver glycogen (percentages)	1.05	6.7
Glucose tolerance	diabetic	nondiabetic

Until now I have observed that metahexamide is tolerated over a sufficiently prolonged period, but not without damage, in doses as low as 100 mg./kg. in rats and in doses as low as 10 mg./kg. in dogs. These doses are 20 times higher than those necessary to produce hypoglycemia in these animals. In personal discussions, other investigators mentioned that they could not make similar observations in their animals. It should be emphasized that these differences in findings may result from the fact that different strains of the same species were used by the various investigators. The decisive trials with low

TABLE 9
METAHEXAMIDE IN DOGS

Dose	(mg./kg., orally)		
	10	25	50
Duration	2 months	3 weeks	1 week
General tolerance	0/5 died, 1 animal sacrificed, anorexia	2/5 died, anorexia	after 7 days, 5/5 in poor general condition, 1 animal sacrificed
Liver	fatty + (retention of bile as in ascending cholangitis) cirrhosis	fatty ++ cirrhosis	fatty +++
Kidney	nephrosis	nephrosis	nephrosis
Heart	without pathological findings	diff. fatty + infiltration	diff. fatty + infiltration
Blood	Ery., Leuk., Hb, Diff. count.: normal	normal	—
Urine	— bile ±	protein + bile +	protein ++ bile +
Glucose tolerance	diabetic	diabetic	—

dosages in rats and dogs are under way, but are not yet finished. Therefore, a final evaluation from the toxicological point of view cannot be made at the moment.

Findings observed in animals thus far, however, indicate that clinical trials should be performed with particular caution and criticism, and over a sufficiently extended period of time. All possible advantages of a drug should be regarded as secondary to the patient's safety. Increased toxicity should never be the price paid for a reduced dose.

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ROLE OF ORAL BLOOD SUGAR–LOWERING AGENTS IN THE MANAGEMENT OF DIABETES

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Four short years span the introduction and extensive clinical trial of sulfonylurea and biguanide compounds as oral blood sugar–lowering agents. The term "hypoglycemic" will be used sparingly in describing their ability to lower blood sugar because in responsive diabetic patients their use is directed toward the attainment of blood sugar levels that are within the normal range (normo- or euglycemic). Since the sulfonylureas and biguanides are not insulin and do not *directly* produce metabolic effects comparable to those that follow insulin administration, the clinician may simply look upon their actions as those by which a certain minimum of insulin, available endogenously or exogenously, appears to be more effective.

The steady stream of pills available to thousands of diabetics has been composed of 4 sulfonylurea and 3 biguanide compounds. With the possibility that additional oral agents may appear on the therapeutic scene, current evaluation is forced into a stern appraisal of the ultimate role of all oral agents in diabetic management. Ideally, a single agent or group of agents, in the absence of significant toxicity, will offer some positive gain through improved or simpler management without jeopardizing the many victories won for the diabetic in 35 years of experience with insulin. The major life-preserving demands of diabetic treatment are already well served by insulin, so that if toxicity in any of the currently available oral agents is to be acceptable it must be at a level of incidence and severity much lower than if there were no other means of treatment.

Sulfonylurea Compounds

The sulfonylurea compounds have undergone sufficient clinical trial to demonstrate their potent blood sugar–lowering action in selected diabetic patients. Significant toxicity in 5.4 per cent of 7193 patients[1] eliminated carbutamide (BZ55*) from further clinical trial in the United States more than 2 years ago, but the relatively nontoxic tolbutamide (Orinase†) has gained ever-increasing clinical application (FIGURE 1). Currently the newer agents, chlorpropamide and metahexamide‡ (FIGURE 2), because of enhanced potency appear to have broadened the field of sulfonylurea usefulness in responsive diabetic patients.

Acute hypoglycemic potencies of metahexamide,[2,3] chlorpropamide (Diabinese§),[4] carbutamide, and tolbutamide are estimated as 4:2:1:<1. Absorption following oral administration of sulfonylureas occurs in less than 4 hours but is most rapid for metahexamide.[5] Delayed excretion and durations of action for chlorpropamide (half life, 35 to 40 hours[6]) and metahexamide (half life, ±30 hours[5,7]) that are longer than those noted for tolbutamide (half life, ±6 hours) further increase the potency of the former when used in maintenance studies.

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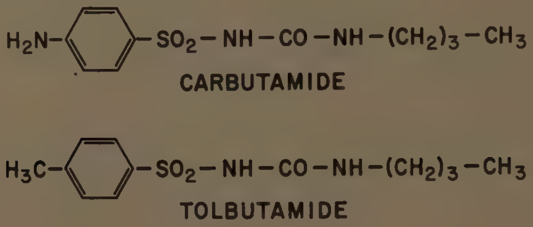
* Eli Lilly and Company, Indianapolis, Ind.

† The Upjohn Company, Kalamazoo, Mich.

‡ Removed from clinical trial in May 1959 because of serious hepatic lesions apparently due to the metahexamide.

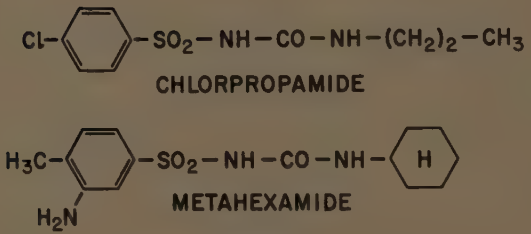
§ Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Mechanism of Action. Little or no evidence suggests that the sulfonylureas lower blood sugar by a direct, insulinlike action. However, ample data support the concept that they act on the pancreas to stimulate the production or release of insulin [8,9] and on the liver to inhibit glucose formation or release.[10,11] The continuing controversy concerning the relative contributions of these two effects of the sulfonylureas indicates that much has yet to be learned concerning their mechanism of action. However, in terms of their clinical application the demonstration of pancreatic islet stimulation[12,13] and of promotion of endogenous insulin secretion by sulfonylurea compounds has given considerable impetus to their extensive trial in many thousands of diabetics.


CARBUTAMIDE

TOLBUTAMIDE

FIGURE 1.


CHLORPROPAMIDE

METAHEXAMIDE

FIGURE 2.

Although some effects of tolbutamide on protein and lipid metabolism are similar to those produced by insulin, such as decrease in blood nonesterified fatty acids[14] and serum amino acids[15] in humans who show a fall in blood sugar after drug administration, more study is needed to determine the extent to which response of total diabetic metabolism to sulfonylureas is comparable to that obtainable with insulin.

Sulfonylurea Compounds in the Management of Diabetes

Successful management of diabetes with any sulfonylurea compound will depend in large measure upon proper selection of patients for trial, and on education of the selected individuals concerning the importance of diet and long-range aims in total treatment.

Selection of patients. Patients responding adequately to a sulfonylurea continue to be those with maturity-onset, stable, so-called "mild" diabetes. In general, "severity" of diabetes is greater the earlier the age of onset,[16] so that selection of diabetics according to onset later in life (age 40 and older), as well as upon insulin requirement (40 U. and preferably 20 U. per day or less) becomes a guide to "mildness" that has far greater accuracy than attempting to substitute a sulfonylurea for a given number of units of insulin. By doing so, many physicians would avoid the trap of treating the early juvenile case in partial clinical remission only to see the apparently beneficial effects swept aside in the ensuing weeks and months as the juvenile pattern continues its natural course[17] and the remission ends.

My colleagues and I have experience with a number of patients under age 40 at onset who were first seen with grossly uncontrolled diabetes after many months of inadequate tolbutamide response. Several were in ketoacidosis, 1 with coma. Two young men, ages 21 and 23 at onset of diabetes, have come for treatment in the past 2 months after ineffective diet and tolbutamide therapy. Each had lipemia retinalis, and 1 came for diagnosis of typical xanthomatosis. Until shortly before admission both stated they felt well and denied diabetic symptoms of sufficient severity to cause undue concern. Serum from each young man was creamy. Laboratory findings confirmed uncontrolled diabetes, mild to moderate ketoacidosis, and severe disturbances in lipid metabolism (TABLE 1).

TABLE 1

ADMISSION FINDINGS IN TWO DIABETICS NOT RESPONDING TO TOLBUTAMIDE

Patient	Sex	Age at onset of diabetes (years)	Duration of treatment with tolbutamide (years)	Blood sugar (mg. per cent)	CO_2 (mEq./l.)	Blood cholesterol (mg. per cent)	Blood cholesterol after insulin (mg. per cent)
V.C.	M	23	1.5	400	16	1275	768*
W.M.	M	20	1	375	25	2066	206†

* One week.
† Six weeks.

These young patients illustrate not so much any directly unfavorable effects of tolbutamide as they do its unrealistic and unwise clinical application, even when a sense of well-being is claimed.

Sulfonylureas are also inadequate during ketoacidosis and coma, infections, and surgical procedures. Emotional upsets and occasional dietary indiscretions may contribute to ineffectiveness, especially when the patient's previous response has been of a borderline character. Exceptions to these limitations unquestionably occur, since the greater a patient's response the more intense must be an upset sufficiently acute to produce uncontrolled diabetes. Not infrequently a sulfonylurea may be successfully resumed when the acute upset is entirely subsided.

Responsive diabetics with established vascular lesions have been treated. However, it has seemed unwise to administer any sulfonylurea when neuropathy or retinopathy are present in an active phase. Where these complications have been present for some time and are stable, their presence in the responsive patient has not been a contraindication. In that age group of diabetics who respond best to sulfonylureas, atherosclerotic occlusive disease of coronary, peripheral, and cerebral arteries is far more common. For individuals with such conditions these agents have seemed desirable because the blood sugar often can be kept normal or nearly so without the concern felt by many that insulin-induced hypoglycemia would produce myocardial infarction or aggravate injury to an already ischemic brain.

Although increasing duration of diabetes has been associated with increased incidence of failure, a number of diabetics of more than 10 years' duration have responded well. No specific duration can be used reliably to demarcate the successes and failures.

Certain patients selected by the above criteria are not controlled by sulfonylureas. The safest means of eliminating all "immediate failures" (those diabetics rapidly developing uncontrolled diabetes and ketoacidosis when insulin is omitted for more than 24 hours[18]) and most "primary failures" (those occurring in the first month of treatment) is to measure the blood sugar response at ±4 hours following a single, adequate loading dose of sulfonylurea.[19]

The "sulfonylurea response" test is by no means infallible. About one fourth of the diabetics having inadequate drop in blood sugar will be found, many days or weeks later, to be doing well on maintenance therapy. On the other hand, later "fair" results or "secondary" failures (after one month of sulfonylurea treatment) occur more often in patients with borderline responses to the test.

Chlorpropamide does not yield consistent reductions in blood sugar level within the number of hours considered reasonable for testing. Therefore, trial with this agent is based on day-to-day maintenance treatment. Metahexamide thus far has yielded surprisingly consistent blood sugar reductions in the responsive patient 4 to 6 hours after administration of the loading dose.

Evaluation of treatment. Standards used in evaluating degree of control obtained with any sulfonylurea will influence significantly the degree of success reported. The seemingly rigid criteria originally adopted for study of Joslin Clinic patients taking carbutamide[20] are equally worthwhile in evaluating tolbutamide (TABLE 2). Although arbitrarily chosen, these strict standards are not meant to create an insurmountable path to success for the sulfonylureas. There are three important reasons for their use:

(1) Early in the course of sulfonylurea trials it was found that the *responsive* diabetic would have blood sugar and urine values consistently in or near the normal range; this has continued to be true.

(2) Patients not qualifying by these criteria often profess subjective well-being, yet increasing tolbutamide dosage, or omitting it entirely, frequently produce no change in blood sugar levels; this suggests that tolbutamide has no significant effect in these individuals.

(3) In the absence of other means of demonstrating antidiabetic action—that is, prevention of late complications—standards of metabolic control should be strict.

TABLE 2
CRITERIA OF CONTROL*

Relation to food	Degree of control†		
	Good Blood sugar‡ mg./100 cc.	Fair Blood sugar‡ mg./100 cc.	Poor
Fasting	110	130	All others
1 hour, p.c.	150	180	
2 hours, p.c.	130	150	
3 hours, p.c.	110	130	
Urine sugar in 24 hours	2 gm. or less	5 gm. or less	

* From Marble and Camerini-Davalos.[19]

† For the purpose of classification as to degree of control, 70 per cent or more of the values must conform with the standards listed in the table.

‡ Glucose as determined by the Somogyi-Nelson procedure.

TABLE 3
RESULTS OF LONG-TERM USE OF TOLBUTAMIDE

	No. of patients	Percentages of total
Good control of hyperglycemia and glycosuria	491	55.0
Fair control of hyperglycemia and glycosuria	121	13.6
Primary failures (within first month of treatment)	199	22.3
Secondary failures	63	7.1
Other	18	2.0
Total	892	100.0

Long-Term Use of Sulfonylurea Compounds

Tolbutamide. Since tolbutamide (Orinase) is the only sulfonylurea having extensive clinical trial for more than one year in the United States, current observations concerning the potential long-range value of sulfonylureas will necessarily be based upon studies with this agent.

Experience of the Joslin Clinic dates from February, 1956, and now includes a total of 1500 diabetic patients, of whom an estimated 1000 have been on maintenance studies for longer than 1 month to more than 3 years. Although final data for this series have yet to be assembled, it is expected the results will be comparable to those reported by Marble[21] in 892 selected diabetic patients of whom 847 were more than 40 years old.

On the basis of the metabolic standards cited earlier (TABLE 2), "good" control has been obtained in 55 per cent and fair control in 13.6 per cent of the patients, giving a total of 68.6 per cent showing satisfactory results with tolbutamide (TABLE 3).

Chlorpropamide. Chlorpropamide now has been given to 122 diabetic patients of the Joslin Clinic since April, 1958. They were selected according to the same criteria used in trials with any sulfonylurea agent, except that a few were chosen because of failure to respond to tolbutamide. In the first 78 patients studied by Hadley *et al.*,[22] the same or a slightly higher percentage of the maturity onset type of diabetic patients achieved adequate diabetic control. Their preliminary report demonstrated good control in 68 per cent and fair control in 15 per cent of the patients so selected.

The current status of chlorpropamide indicates some decrease in favorable results since these earlier observations (TABLE 4). Good or fair control is being obtained in 55 per cent, but some of the patients were those who failed to respond to tolbutamide. A few have improved following administration of chlorpropamide, but the majority fail to improve sufficiently to be classified as successful.

TABLE 4

RESULTS OF MAINTENANCE STUDIES WITH CHLORPROPAMIDE* IN 122 DIABETICS

	No. of patients	Percentages of total
Good control and fair control	67	55
Insufficient trial	28	23
Primary failures	17	14
Secondary failures	4	3
Side effects	6	5
Total	122	100

* April, 1958, to April 1, 1959.

TABLE 5

RESULTS OF MAINTENANCE STUDIES WITH METAHEXAMIDE*

	No. of patients	Per cent of total
Good control and fair control†	55	65
Primary failures	19	22
Discontinued—side effects	7	8
Insufficient trial	4	5
Total	85	100

* August, 1958, to March 25, 1959.
† After 1 to 8 months.

Metahexamide. Results obtained with metahexamide from August, 1958, to March, 1959, in 85 diabetics show success comparable to that found with tolbutamide and chlorpropamide (TABLE 5). The rate of primary failure was about 25 per cent, but a number of the patients treated were chosen because of failure to respond to tolbutamide. Of 26 such individuals, 8 promptly failed to respond to metahexamide and 18 were under adequate control. A few diabetics responding well to tolbutamide were transferred to metahexamide and obtained equally good blood sugar results with much smaller doses taken once daily.

Toxicity of Sulfonylurea Compounds

Tolbutamide. Tolbutamide administration has been associated with remarkably few untoward effects. Although treatment was discontinued in 9 of the 892 patients reported by Marble because of allergic skin rashes in 6 and a variety of mild upper gastrointestinal symptoms in 3, none of these upsets was severe and in most instances their relation to the drug was questionable.

Serious toxicity possibly related to tolbutamide has occurred in 4 patients. In 1 woman with pre-existing cirrhosis and esophageal varices, jaundice lasting 2 weeks appeared 3 months after tolbutamide was begun. A second woman, age 59, taking the drug for approximately 12 months developed a comatose state and died with typical hepatic failure. Postmortem examination showed mild portal cirrhosis of some duration and a superimposed acute hepatitis, suggesting infectious origin. These changes were interpreted* as being not comparable to those seen in a case of liver damage apparently due to carbutamide.

With far better documentation of its relation to tolbutamide, an allergic type of purpura occurred in a 63-year-old man and a 56-year-old woman. After taking the drug for 1 and 6 months, respectively, they developed lassitude, petechial eruptions of the extremities, fever, and hepatosplenomegaly. A reduction in platelets, elevated Bromsulphalein retention and alkaline phosphatase levels and, in one instance, fatty infiltration of the liver in tissue obtained by needle biopsy were found. Studies to determine the presence of antiplatelet agglutinins were performed† on the sera from these patients. The results, to be reported by Mirsky and Krall,[23] indicated that antiplatelet antibody formed in the presence of tolbutamide produced the clinical picture. Administration of hydrocortisone and omission of tolbutamide produced rapid recovery in the male patient and subsequently it became possible gradually to omit hydrocortisone without recurrence. The woman's symptoms and signs disappeared gradually after omission of tolbutamide.

No instances of granulocytopenia have been demonstrated. Results of extensive liver function studies carried out serially in large numbers of patients receiving tolbutamide are summarized by Mehnert *et al.*[24] No consistent or important abnormality was found, except in the alkaline phosphastase levels, which at about 1 month after therapy was started showed an average rise of 1 Bodansky unit. This rise was often maintained, but seldom rose further and often returned to normal despite continuation of tolbutamide.

Duration of tolbutamide administration in a large number of patients is now sufficiently long to warrant considerably increased confidence in the drug's safety, since untoward effects requiring cessation of the drug are anticipated in our experience in 1 per cent of the cases or less. C. J. O'Donovan‡ has stated that in more than 9000 diabetics treated with tolbutamide for 6 to more than 28 months, only 1.53 per cent experienced untoward effects requiring omission of the drug. No deaths clearly attributable to tolbutamide have been reported, and in our own experience patients who died of other causes and who were autopsied, on histological examination were found to have no lesions of any organ that would suggest tolbutamide toxicity.

Chlorpropamide. Serious or prolonged hypoglycemia can be avoided by proper dosage. Occasional nausea, vomiting, epigastric distress, generalized weakness, and dizziness occurring singly or together are uncommon since doses of chlorpropamide exceeding 500 mg. daily have been discontinued.

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† By James Tullis and Hugh Pyle, Protein Foundation, Boston, Mass.

‡ Department of Clinical Investigation, The Upjohn Co., Kalamazoo, Mich.

Allergic skin reactions have been seen in at least 2 patients, but they were not severe and disappeared promptly on discontinuance of the drug. One man developed jaundice 3 weeks after treatment was started. Tissue removed by needle biopsy of the liver revealed intracanalicular bile stasis and minimal hepatic cellular damage. Gradual recovery took place after cessation of drug therapy.

No instance of leukopenia, granulocytopenia, or decrease in platelets has been noted to date. Liver function tests have yielded results comparable to those found with tolbutamide.

Although no cumulative report of chlorpropamide toxicity based on combined experiences is currently available, there were at least 5 instances of jaundice reported at a conference on chlorpropamide and diabetes mellitus in September, 1958.* Some of the cases of toxicity appeared to be related to administration of chlorpropamide in dosages that now seem excessive.

Metahexamide. Metahexamide in dosage of 300 mg. per day or higher frequently produces epigastric distress that is usually relieved by reduction in dose. However, persistence of these symptoms forced discontinuance of the drug in 4 patients.

Three additional diabetics were noted to have abnormal liver function tests while taking metahexamide. One woman developed epigastric distress and jaundice during the second week after treatment was started. She received 200 mg. daily for 1 week, at which time liver function tests were entirely normal. During the second week 400 mg. of metahexamide was taken, and at the peak of her jaundice the bilirubin was 9.4 mg. per cent and serum transaminase, 414 U. Following cessation of drug treatment this woman gradually improved and at the end of 6 weeks, liver function tests were approaching normal. Another patient, who had had jaundice after taking carbutamide, developed minimal jaundice and abnormal liver function tests after metahexamide. A third diabetic was found to have abnormal liver function at the time metahexamide was started, but the tests have shown an improvement despite continued administration of the drug.

Kirtley reported† that of 1646 diabetics receiving metahexamide as of February 26, 1959, 5 developed jaundice on doses of 50 to 250 mg. per day and 1 while taking 600 mg. per day. Two others who became jaundiced following metahexamide were reported; one of these had an associated hemolytic anemia.[25]

Biguanide Compounds

Biguanide compounds are capable of exerting potent blood sugar–lowering action in all types of diabetics and have thus, quite naturally, created considerable furor regarding their ultimate role in diabetic management—in particular, with respect to their possible value in replacing insulin in younger and more severe cases. The brief presentation below of the Joslin Clinic experience with the biguanides when supplemented by the more detailed account by Krall,[26] will indicate the extent to which such optimism necessarily must be limited.

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* Sponsored by The New York Academy of Sciences and Chas. Pfizer & Co., Inc.

† At the Conference on Insulin and the Oral Hypoglycemic Agents, Indiana University Medical Center, Indianapolis, Ind., sponsored by Eli Lilly and Company, February 27 to 28, 1959.

In the United States clinical trials with the biguanides, now conducted more than 2 years, are second in duration only to tolbutamide among the oral agents available. Although they have now been given to approximately 5000 diabetics, their clinical use has been in the hands of relatively few investigators and there has not occurred a rapid extension of their administration to many thousands of patients such as that following the introduction of the sulfonylureas.

Since both laboratory and clinical data concerning biguanide compounds are based primarily upon the use of the phenethylbiguanide (phenethyl-formamidinyliminourea or Phenformin) known simply as DBI* or PEBG (FIGURE 3); the term biguanide or DBI will be used in describing the actions of any 1 of the 3 biguanide analogues now available. The other 2 analogues are the normal amyl (DBB)* or normal butyl (DBV)* biguanides. The 3 preparations appear to vary only in their degree of effectiveness in lowering blood sugar and in their tendency to produce gastrointestinal side effects, so that principally the biguanide structure is involved in their actions.

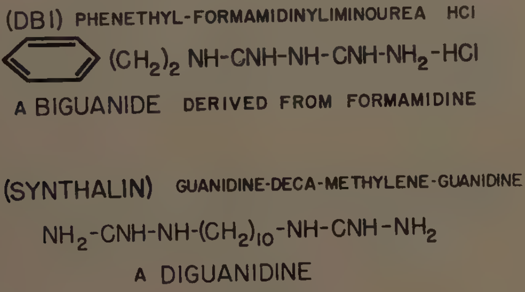
(DBI) PHENETHYL-FORMAMIDINYLIMINOUREA HCl
(CH2)2 NH-CNH-NH-CNH-NH2-HCl
A BIGUANIDE DERIVED FROM FORMAMIDINE
(SYNTHALIN) GUANIDINE-DECA-METHYLENE-GUANIDINE
NH2-CNH-NH-(CH2)10-NH-CNH-NH2
A DIGUANIDINE

FIGURE 3.

Metabolic effects of the biguanides. The ability of DBI to lower blood sugar in diabetic humans has been amply confirmed,[27] but failure to do so in normal humans[28] is unexplained.

In addition to lowering blood sugar in normal, alloxan diabetic,[29] depancreatized, eviscerated, and hepatectomized animals,[30] as well as in diabetic humans, DBI produces a number of metabolic effects that have been summarized elsewhere;[31,32] these effects, in part, are as follows: (1) increased glucose uptake in rat diaphragm and liver, despite apparently decreased glucose oxidation; (2) no formation or possible depletion of glycogen in muscle and liver; (3) absence of hyperglycemic response to epinephrine and glucogon; (4) elevation in the blood of the diabetic of the lactate, pyruvate, and citrate levels; (5) a fall or no change in hepatic vein-glucose output; and (6) the occurrence of gastrointestinal side effects, dosage-related and apparently not of local origin, with anorexia, nausea, vomiting, and diarrhea.

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* U. S. Vitamin Corporation, New York, N. Y.

The mechanism by which these metabolic effects are produced and blood sugar lowered appears to be that of increased anaerobic glycolysis, occurring as a result of decreased glycolysis via the oxidative pathway of the tricarboxylic acid (Krebs) cycle. Evidence of interference with energy transfer due to a block or partial block in the oxidative pathway has been reported by Wick *et al.*[33] and by Steiner and Williams.[34] However, the site of action of the biguanides is still controversial, particularly with regard to (1) the relative contributions of peripheral tissues and liver to the glycolysis induced and (2) the possibility that in diabetic humans, glycolysis via the hexose monophosphate shunt may be increased.[35]

Pharmacology. Clinical observations suggest that biguanides are rapidly absorbed, but no data are available giving measurements of these compounds in tissues or blood. Measurable blood sugar lowering occurs within 2 to 3 hours in many instances, but duration of action appears to be 8 hours or less. No other pharmacodynamic effects of DBI have been observed.

Biguanides in the Management of Diabetes

Joslin Clinic patients were first given clinical trials with DBI in December of 1956. The current series, carefully followed and studied under the supervision of Krall, now totals more than 350 individuals. However, such numbers of diabetics do not represent wholesale selection for trial, since they have been carefully culled from approximately 10,000 diabetic admissions to the New England Deaconess Hospital.

Blood sugar lowering. Early in the clinical trials with DBI it was extremely difficult to demonstrate significant reduction of hyperglycemia comparable to that produced by insulin or, in the responsive patient, by a sulfonylurea. This is accounted for by two observations:

(1) The effect on blood sugar is in most instances gradual as dosage is increased, and attempts to give a large loading dose of a biguanide is frequently followed by gastrointestinal side effects.

(2) All patients appear to need some insulin, either endogenous or exogenous in origin, in order to obtain continuing regulation with biguanide compounds.

Therefore, when insulin is omitted or significantly reduced in the more severe cases a number of hours before administration of a biguanide, the momentum of the uncontrolled diabetes is sufficient to make the compound ineffectual. In such instances insulin must be given promptly to prevent serious ketoacidosis.

Selection of patients. Short of clinical trial, no definite clue is available to determine which diabetic patients will do well. Diabetics of all ages, durations of diabetes, and previous insulin dosages have been studied. However, during ketoacidosis, infections, surgery, and periods of severe stress, the biguanides almost invariably have been replaced by insulin.

Results. Results with biguanides given for periods as long as 26 months, in studies heavily weighted with severe cases, continue much the same as those in 173 patients previously reported by Krall and Bradley.[27] Blood sugar lowering in the earlier series was demonstrated in 62 per cent (107) without significant side effects and in 26 per cent with dosages producing gastrointestinal symptoms of sufficient severity to warrant discontinuing the drug. In

12 per cent of the patients the side effects occurred so early that dosage sufficient to produce adequate reduction of hyperglycemia could not be tolerated. Nineteen of 31 diabetics classed as failures with sulfonylureas responded without significant side effects.

Maintenance studies. In the 107 diabetics obtaining adequate response to biguanides without side effects, 64 (60 per cent) were rated as having good control by the strict metabolic criteria cited above (TABLE 6). An additional 31 patients (29 per cent) had fair control and the rest rated as poor.

The striking feature in diabetics treated with DBI plus insulin has been stabilization of the diabetes in many individuals, of benefit particularly in the extremely unstable, often long-duration case having severe insulin reactions and threatened with loss of employment. Approximately 30 of these unusually severe cases have been dramatically improved. Despite the maintenance of remarkably good blood and urine sugar values, severe hypoglycemic reactions have been virtually eliminated in these patients.

With biguanides, many patients are managed on insulin doses that are 20 to 50 per cent lower than those used before their administration. In a few instances patients on biguanides alone or with insulin will, after days or weeks or months, develop lethargy, weight loss, and lack of well-being. Addition of insulin or a slight increase in dose rapidly has restored these individuals to normal.

TABLE 6

RESULTS OF MAINTENANCE STUDIES WITH BIGUANIDES

	Number of patients	Percentage of initial successes	Percentage of all patients tried
Good control of hyperglycemia and glycosuria	64	60	37
Fair control of hyperglycemia and glycosuria	31	29	18
Poor control of hyperglycemia and glycosuria	12	11	7
Total	107	100	62

Side effects. Anorexia, nausea, gas, metallic taste in mouth, vomiting, and diarrhea always occur when dosage of biguanide is raised far above hypoglycemic levels. They are classed as side effects because they are always rapidly and completely reversible within 24 hours after omission of DBI. Side effects, if severe, can contribute to the risk of ketoacidosis, so that a supplementary dose of crystalline insulin may be needed. In most instances DBI can be resumed at the meal following manifestation of the side effect or the one following, but in reduced dosage.

Toxicity of the biguanides. In striking contrast to the toxic effects reported to occur in the liver and kidneys following administration of Synthalin is the absence of demonstrable injury to the hematopoietic system, liver, or kidneys of animals[36] or humans [37,38] following biguanide administration. However, Creutzfeldt and Moench[39] after intravenous administration of phenethylbiguanide to guinea pigs in much larger doses than those used orally in earlier toxicity studies, have observed certain changes in the liver and kidneys resembling in mild form those found after administration of Synthalin.

In the Joslin Clinic experience, toxicity has been remarkable for its absence. Three patients dying of conditions unrelated to their biguanide ingestion showed no histological abnormalities at autopsy that could be attributed to the drug.

Combinations of Blood Sugar-Lowering Agents

Combinations of insulin and tolbutamide have been reported by Fabrykant[40] to be effective in 9 labile and 28 stable but insulin-dependent diabetics. Improved stability was noted in the unstable cases and improved regulation with lowered insulin dosage in those who were stable.

Our experience with either of these techniques is extremely limited. As to value in the unstable diabetic, one may suppose that any effect gained by the use of tolbutamide would operate through the liver in reducing glucose output, since such patients appear to have little, if any, insulin whose secretion might be stimulated. On the other hand a few patients with maturity-onset, stable diabetes responding inadequately to tolbutamide have obtained better blood sugar control when a reduced dose of insulin was added than was produced by insulin alone.

Beaser[41] administered tolbutamide or chlorpropamide with a biguanide to diabetics responding inadequately to a sulfonylurea or biguanide alone. He obtained an additive effect in three and complete replacement of insulin in two patients. A similar technique has been tried in nine of our patients with good control resulting in four, control comparable to that with insulin alone in three, and failure in two.

Discussion

What benefits should oral agents provide? Oral blood sugar-lowering compounds are here to stay for at least the next few years. Not yet available are the truly long-term studies of 10 to 15 years or more needed to evaluate the effects of sulfonylureas and biguanides upon late neurological or vascular complications. Acceptance and use of these agents in diabetics indicates a willingness to undertake the calculated risk that they will not affect victories won for the diabetic[42] through the judicious use of insulin during the past 37 years. Therefore, clearly demonstrable benefits in day to day management of diabetic metabolism in the absence of significant toxicity should offer: (1) better management of diabetes than has been obtainable with diet or with diet and insulin, and (2) demonstrable reduction of hyperglycemia and glycosuria of sufficient degree and duration that control of the diabetes is comparable, and preferably superior, to that obtainable with diet, insulin, or both.

Such stringent criteria are warranted in today's new era of diabetic treatment because, although numerous, the available blood sugar–lowering agents are as yet sufficiently limited to particular segments of the diabetic population that they may be termed specialized or precise tools in treatment. Only by recognizing these limitations and insisting upon good metabolic standards will the physician fulfill his role in protecting his patient from the acute emergencies of diabetes and, in the long run, from late complications. The fact that in selected patients both sulfonylurea and biguanide do qualify according to these criteria is the principal reason for their currently expanding role in diabetic treatment.

After having accepted continuing and adequate blood sugar-lowering action as a temporary standard that indicates that oral agents may be antidiabetic in selected patients, the physician should be guided further by the principles outlined below.

General principles. Patients receiving any oral agent must be followed closely and examined regularly, and blood sugar tests should be done at random or 2 to 4 hours postprandially. Particular attention should be paid to evidences of toxicity and to the premature development of complications such as neuropathy, infections, hepatomegaly, and vascular lesions. All diabetics should be trained in the principles of diet and the general measures needed to protect them from acute emergencies.

Insulin must be started or dosage increased promptly if blood and urine sugar values are not within or approaching the limits outlined above, particularly during ketoacidosis, infections, surgery, or other stressful situations.

Diet is vital to the success of any oral agent and in obese individuals is best monitored by observation of changes in body weight. Although it has been claimed that a large majority of obese diabetics can be controlled by diet alone[43] and that the unnecessary use of insulin or oral agents may promote weight gain, in our experience a significant number of obese patients not responding to a strict diet in a few days to two weeks promptly improved when an oral agent was added. As time passes and sizable reductions in weight occur, some of these individuals can be taken off the oral agent without a recurrence of hyperglycemia and glycosuria. In others, the oral compound must be resumed, usually followed by prompt return to normal of blood and urine sugar values.

Remission is reported to occur in 1 of 3 diabetics with juvenile onset (age 15 or under)[44] and in rare instances may be temporarily complete.[45] Adults with maturity-onset type of diabetes also may experience striking remissions,[46] to the extent that the condition may be difficult to demonstrate within a few weeks after diagnosis and the exact age at onset notoriously uncertain. In such individuals treatment successes should not be attributed to oral agents.

Sulfonylurea compounds. Age at onset of diabetes will be the single most reliable clue to the long-range effectiveness of the sulfonylureas. Although certain young patients (onset under age 40) have diabetes that reacts like that of stable, maturity-onset cases and that may respond adequately at first, the chance of continuing response is sufficiently small that they should receive one of these agents only rarely, if ever. All unstable diabetics, regardless of age, are not suitable candidates unless it can be shown that stabilization is attained. This has not been our experience.

The sulfonylurea response test will eliminate all immediate and most primary failures. It is a desirable safeguard in the hands of physicians not accustomed to treating large numbers of diabetic patients and has special value in cases with a borderline status determined by age at onset, insulin dose, and stability of diabetes.

The incidence of primary failures rises rapidly in those individuals requiring

30 to 40 U. of insulin or more per day. Secondary failures will vary from 5 to 8 per cent and are not clearly related to previous insulin dose. About half are explainable by diet excess or a variety of stressfull situations, and the remainder are idiopathic. The delayed failures are of special importance because of the possible implication that islet-cell exhaustion may be produced through continuing sulfonylurea stimulation. This potential disadvantage of these oral agents has not thus far been supported by laboratory or clinical observation. In the Joslin Clinic experience the secondary failures (7.1 per cent) have not required larger doses of insulin or been more difficult to manage when insulin was resumed. There is, however, a brief period during which higher dosage may be needed to overcome the uncontrolled diabetes.

The roles of chlorpropamide and metahexamide will be determined by the same general principles governing the use of tolbutamide, but are currently modified as follows.

(1) Since they are more potent, chlorpropamide and metahexamide will elicit adequate response in a few of the diabetics inadequately controlled with tolbutamide; however, it is to be expected that some of these patients will become secondary failures.

(2) Longer duration of action allows chlorpropamide and metahexamide to be effective when given in a single dose daily or once in two days.

(3) Offsetting the above two advantages is the question of safety. Although no deaths based on idiosyncratic reaction to these drugs have been proved, their administration has been associated with a disturbing incidence of jaundice, which thus far has been reversible on cessation of chlorpropamide or metahexamide. Concern over the ultimate toxicity of chlorpropamide and metahexamide is not fully eased by the observation that jaundice and other less significant dermatological and gastrointestinal symptoms have occurred primarily when dosage of these agents is excessive.

All sulfonylureas have a maximally effective dosage level beyond which further significant blood sugar-lowering action cannot be obtained.

Despite the limited application of sulfonylureas to selected diabetic patients and the precautions needed to ensure their safety, their blood sugar-lowering action has given the diabetic several striking benefits: (1) reduction of hyperglycemia and glycosuria, apparently attributable in large measure to increased endogenous insulin production and secretion; (2) regulation of carbohydrate metabolism in the fully responsive patient by strict clinical standards, comparable or superior to that obtainable by diet or insulin; (3) control that is often smoother than with insulin and that is associated with a sense of well-being, lessened hunger, and increased incentive to diet adherence; (4) attainment of precise metabolic control with a much reduced risk of serious hypoglycemia in responsive diabetics with occlusive atherosclerotic lesions; and (5) convenient administration, especially for those who are blind or with handicaps making insulin administration difficult.

Biguanide compounds. The biguanides DBI and DBB, either alone or in combination with insulin, will lower blood sugar in a large majority (approximately 80 per cent) of diabetic patients. However, gastrointestinal side effects occur with these compounds in one-third of the cases, leaving 50 to 60 per cent in whom they may be considered to be effective. Of this group about

90 per cent should attain good or fair control of diabetes according to criteria outlined above, although such results are obtained only in combination with insulin in the majority of diabetics in this series and by maximal cooperation in such matters as diet adherence, adjustment of insulin, and urine tests by all patients. Under these conditions, then, blood sugar lowering can be assisted by the proper use of biguanides in about 50 per cent of patients; thus, the biguanides are an adjunct or a specialized tool in clinical management.

Attempts at stabilization of labile, severe growth-onset diabetics with DBI and insulin is justifiable, but such regulation is best carried out in the hospital. At present the use of biguanides in Joslin Clinic patients is limited primarily to this notoriously difficult group of diabetics because for them the drugs can provide measurable improvement in their present state.

Benefits for young diabetics given DBI alone are limited to those in remission where an effort is being made to sustain the remission beyond the usual 3- to 12-month period,[47] and to patients in partial remission in whom 20 U. of insulin or less may on occasion be replaced. The risk of jeopardizing growth and development by uncontrolled diabetes is great if this relatively small group of young diabetics is not followed with utmost care and if insulin is not promptly resumed when good clinical control is no longer attained.

Biguanides plus diet will adequately control as yet undetermined numbers of milder, maturity-onset types of diabetics. However, many of these respond so well to a sulfonylurea compound (at least partly in response to their own insulin) that biguanides may offer little or no benefit, particularly in view of the need for multiple dosage and the incidence of side effects.

In the maturity-onset cases, especially those of age 40 and more, DBI may effect good clinical control when sulfonylureas have failed. In such patients, and in the few where DBI or a sulfonylurea are adequate only when given in combination, biguanide therapy may be tried. However, such individuals not infrequently fail to experience a sense of well-being—perhaps related to uncontrolled diabetes—and welcome a return to insulin.

Toxicity due to biguanides has not been a clinical problem, despite experimental observations indicating metabolic activities showing striking similarities to those found with Synthalin. Thus, biguanides bear much the same relationship to Synthalin as tolbutamide does to carbutamide.

Combinations of blood sugar–lowering agents. Sulfonylureas combined with insulin appear worthy of further trial in the stable, maturity-onset diabetic, provided that the blood sugar results obtained are superior to those with either agent given alone.

Since administration of a sulfonylurea and a biguanide together is the ultimate in blood sugar-lowering devices to bypass the use of insulin, it appears likely that this technique will have lasting value in only a minority of patients incompletely responsive to the sulfonylureas.

Conclusion

Currently available oral blood sugar–lowering agents are valuable tools capable of supplementing diet and insulin in the total treatment of carefully selected diabetic patients.

Sulfonylurea compounds are effective in the majority of stable diabetics who have an onset age of 40 and over and take 40 U. of insulin or less per day.

Relatively long-term studies with tolbutamide indicate sufficient safety from toxicity and ability to maintain clinical control in responsive diabetics to warrant confidence in its continuing role in management of the disease.

Chlorpropamide and metahexamide are more potent than tolbutamide. They may adequately control some of those patients incompletely controlled by tolbutamide, but their effectiveness in single daily dosage offers an advantage offset by the incompletely resolved question of toxicity. Further cautious clinical trial appears justified.

DBI and its biguanide analogues are capable of lowering blood sugar in many diabetics of all types, but dosage limitations imposed by reversible gastrointestinal side effects restrict their clinical value primarily to stabilization of the extremely severe and labile diabetic. In such patients administration of DBI plus insulin is a particularly refined technique requiring skillful handling.

Our experience with biguanides in milder, maturity-onset diabetics is limited. For patients of this group, who are responsive to a sulfonylurea, it has been the preferred oral agent. In those not responsive to a sulfonylurea the possible role of biguanides appears to warrant further trial.

At present no oral agent is preferred for well-controlled, insulin-dependent diabetics free of severe hypoglycemic reactions.

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AN ASSESSMENT OF ORAL ANTIDIABETIC THERAPY

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When tolbutamide became an established form of oral diabetic therapy, it had to measure up to insulin as a standard for therapeutic comparison. The generally satisfactory experience with tolbutamide now makes this drug the standard by which all other modifications of the original sulfonylurea formula and unrelated compounds must be measured. This paper answers two questions: Do these modifications represent a contribution, and are they safe? In addition an attempt will be made to describe variable responses to the same drug by the same patient.

Variable Drug Responses by the Same Patient

As with insulin, diabetic patients exhibit altering and variable responses to the oral agents according to altering physical stress. Primary and secondary tolbutamide failures have been dismissed dogmatically as being insulin deficient in the physiological sense. That certain emotional and psychological factors in these patients may alter their responsiveness to these drugs is illustrated by the following cases.

FIGURE 1 demonstrates the primary failure of tolbutamide therapy in a 52-year-old woman in whom the daily requirement for 100 U. of insulin could not be influenced in the slightest by maximum amounts of tolbutamide while she was followed carefully in the hospital. It will be noted that several weeks after the suicide of her husband, when the patient eagerly sought a transfer to tolbutamide and overcame all medical resistance to her request, she was able to effect a successful change from her large insulin dose to 1 gm. of tolbutamide, a dose that has been maintained for almost 2 years. No attempt has been made as yet to probe into the psychodynamics of this bizarre phenomenon; the only comment the patient has expressed when questioned as to an explanation for the dramatic success has been "God probably took pity on me."

FIGURE 2 illustrates a cyclical variation of responsiveness in a woman whose introduction to diabetes occurred during a severely agitated depression accompanied by ketosis. Following a period of insulin therapy an effective change to tolbutamide was accomplished. Without explanation a steadily increasing dose of tolbutamide to the point of failure to control the diabetes was required and insulin had to be reinstituted. This physiological alteration accompanied a recurrent depression that responded to electric shock therapy, during which time insulin was continued. Parallel with her clinical improvement the insulin dose was reduced and tolbutamide reinstituted as daily therapy for another one-half year. Twice more this cycle was repeated; each time the first portent of the erupting emotional crises appeared in the failure of the usual small doses of tolbutamide to control the diabetes. By the second and third depressions the psychiatrist had instituted an alarm system whereby any increase of tolbutamide requirement would be reported to him as the sign of oncoming melancholia.

The first patient represents a primary failure and secondary success. The second patient was a primary success and secondary failure on a cyclical pattern. One wonders whether some of the other primary or secondary failures with oral diabetic therapy may be due to a basic emotional dependency upon insulin, injections, and the obvious associated implications of a masochistic need.

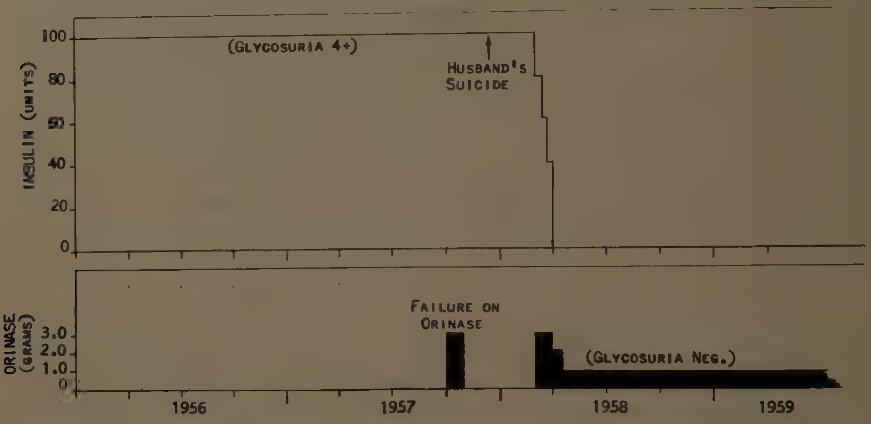

FIGURE 1. Secondary successful response after primary failure. Patient R.F.; female 52 years; D.M., 5 years duration.

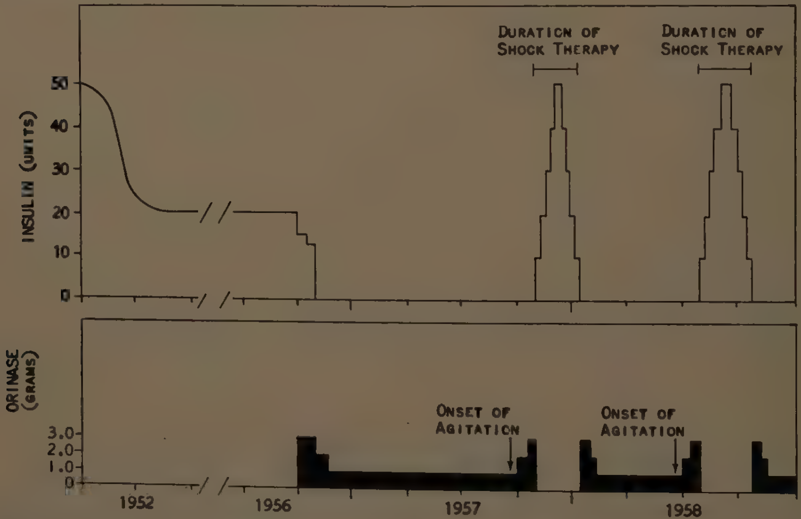

FIGURE 2. Mental depression and the cyclical response to Orinase. Patient S.W.; female, 62 years; D.M. onset in 1952; ketosis and severe depression.

Variations by the same patient in response to different agents is illustrated in the case of a 26-year-old man with hemophilia who developed diabetes 1 year ago. This patient required 60 U. of insulin daily, but control of glycosuria was satisfactory and the patient presented a desperate emotional problem because each injection of insulin produced a huge hematoma. An attempt to achieve replacement of the insulin with tolbutamide failed at the time. With some reluctance DBI was started and doses of 50 mg./day effected a reduction and, finally, abolition of the need for insulin, with excellent control of glycosuria and normal blood sugar levels. Soon after he required 100 mg. of DBI, then 150 mg., and finally 200 mg. which could not control glycosuria or ketonuria without severe nausea and diarrhea. At this time a few days of insulin therapy were effective in obtaining control of the diabetes and a second trial with tolbutamide proved successful. Since that time he has been managed with 0.5 gm. of tolbutamide daily.

Such dramatic instances make it evident that considerable fuzziness and overlapping will characterize any attempt to delineate sharply the areas of action and superiority of one drug over another.

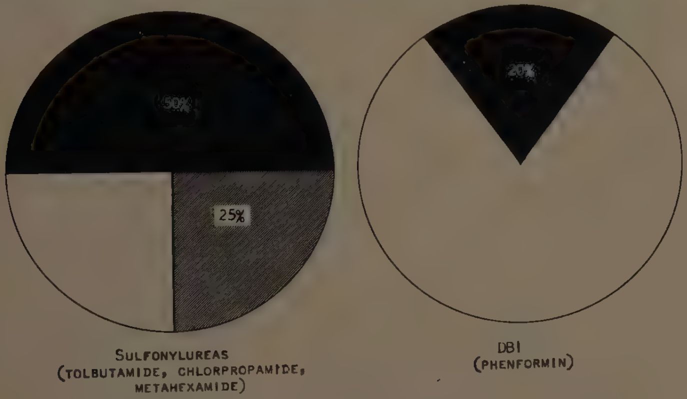

FIGURE 3. Percentage of all diabetics responsive to oral agents.

Contribution or Duplication?

Three sulfonylurea compounds will be compared: tolbutamide (Orinase*), chlorpropramide (Diabenese†), and metahexamide (Melonex‡, Euglycin*). The actions and therapeutic range for all three compounds are identical, as illustrated in FIGURE 3. The only therapeutic differences lie in the relative increased potency of the newer compounds with effective dosage in the range of one tenth (metahexamide) to one third (chlorpropramide) that of tolbutamide. It will be seen that this increase in potency is associated with an inexcusable increase in serious side effects and toxicity especially in liver damage. It stands in marked contrast to the improvement in steroid therapy in which increase in potency has been achieved with decreasing undesirable side effects.

For the patients with maturity-onset diabetes the generally accepted figure of 50 to 75 per cent successful applicability is equally true for each of the sulfonylurea compounds and their similar therapeutic spectrum is illustrated in FIGURE 3. It is apparent that only a fringe of a few per cent may reveal

* Product of The Upjohn Company, Kalamazoo, Mich.
† Product of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.
‡ Product of Eli Lilly and Company, Indianapolis, Ind.

superior results from one of the newer modifications. Limitations in juvenile diabetes, unstable diabetes, and ketosis that have characterized the early experience of tolbutamide therapy hold true in identical fashion for chlorpropramide and metahexamide. Where the former fails the latter two drugs will not succeed so that these newer agents will not reduce the hard core of insulin-deficient patients to any degree.

On the other hand, my own experience indicates that Phenformin is limited in usefulness to less than 20 per cent of the diabetic population. This figure includes a number of mild diabetic patients not requiring insulin in whom asymptomatic glycosuria can respond to a therapeutic, but minimal dosage of DBI. Limitations of therapeutic range will be described in the section of this paper devoted to side effects. I have not been able to duplicate the purported claim that DBI will abolish, reduce, or stabilize the insulin requirements in a single insulin-treated patient. The most encouraging results with DBI have accompanied its use as a supplement to existing tolbutamide therapy in some primary and most secondary failures. Totally depancreatized humans on DBI require biweekly doses of insulin along with DBI. Keeping this in mind and recognizing the possible synergistic insulinlike effect of tolbutamide in such cases of DBI intolerance, it became apparent that it could be useful generally to employ suboptimal doses of DBI, 50 mg./day for example, with accompanying amounts of tolbutamide of between 1 to 2 gm./day. This was begun first with secondary failures on tolbutamide therapy. Secondary failures have been presumed to represent some block by an adaptive mechanism to the action of this sulfonylurea. It has been possible in such instances to regain therapeutic success after a temporary period of insulin therapy, or by replacing the tolbutamide with another sulfonylurea derivative. However, with small doses of DBI it was possible to overcome the failure of tolbutamide in about 150 patients who were able to continue with total oral therapy without any side reactions from DBI. More recently I have employed the same technique in some primary failures with tolbutamide. These are adult patients in whom the 3-gm. maximum dose of tolbutamide proved ineffective after 3 or 4 days in controlling the glycosuria and hyperglycemia. It was found that the addition of 50 mg. of DBI to the tolbutamide dose very often effectively restored satisfactory control. At this point the tolbutamide could be reduced and the DBI discontinued. Whenever the tolbutamide was discontinued in this combined therapeutic approach, glycosuria recurred.

Since all side reactions can be avoided, this technique of combination therapy offers the safest method of DBI administration, in addition to providing a solution for tolbutamide-resistant patients.

Of about 1000 diabetic patients attending the outpatient clinic of the Mount Sinai Hospital, about 200 are treated by mild dietary restriction alone, about 400 require insulin, and the remaining 400 use the oral agents. In the latter group, 329 patients are well controlled by tolbutamide alone, 50 use a combination of tolbutamide with DBI, 19 are on chlorpropamide, and only 3 are left on metahexamide therapy. The disproportionately small number of patients on chlorpropamide and metahexamide represents the unwillingness of the clinic physicians to resort to these more toxic drugs in the face of such overwhelming satisfactory therapeutic success with tolbutamide in the bulk of patients.

Furthermore, the contrast between the safety of tolbutamide therapy and the added complications of constant surveillance of hematological and liver-function studies necessary with chlorpropamide and metahexamide treatment made the former agent most desirable and economical for the patients and simplest for the physicians. In private practice, roughly the same distribution is noted with about one half the patients on tolbutamide and the remainder use insulin. Combination therapy with either sulfonylurea compounds or DBI and insulin has proved useless not only as a therapeutic failure, but also because it is anxiety-producing for both patients and physicians.

Side Effects and Toxicity

In May 1957, at a meeting of the Food and Drug Administration at which the public release of tolbutamide was being considered with some hesitancy, Perrin H. Long stated that his vast experience with thousands of analogues of sulfonamide type led him to expect no toxic effects when the compound contained a methyl group in the para position of the benzene ring. This prediction has since been substantiated by the insignificant number of side effects or toxic reactions due to tolbutamide therapy. More important than frequency should be the nature of the adverse and undesirable reaction associated with a drug. It is evident that conditions such as liver injury carry greater weight than do skin rashes in analyzing comparative toxicological data. TABLE 1 illustrates the presence or absence of certain serious side effects and toxic reactions associated with each of the oral agents.

TABLE 1

COMPARISON OF SIDE EFFECTS AND TOXICITY OF ALL ORAL AGENTS

Drug	Dosage Range in mg.	G.I. intolerance	Liver damage	Severe hypoglycemia	Acetonuria	Neurological effects
Tolbutamide..........	500–3000	0	0	0	0	0
Chlorpropamide......	250–1000	+	+	+	0	+
Metahexamide........	50–250	+	+	0	0	+
DBI (Phenformin) ...	100–150	++++	0	0	+	0

Gastrointestinal tolerance for tolbutamide is so high that the therapeutic ceiling of 3-gm. maximum can be exceeded manifold without distress. In striking contrast every one of the other three agents has a distinct maximal tolerated dose that often is associated with an inability to tolerate it. In other words, 1 gm. chlorpropamide, 300 mg. of metahexamide, and 150 mg. of DBI produce nausea and pyrosis in 25 to 50 per cent of the patients in our series. DBI, unfortunately, is limited in obtaining full therapeutic effects because of the inherent tendency to affect the central nervous system center for nausea and vomiting, whenever the therapeutic dose is reached, in more than one half of the patients. This untoward effect is even more undesirable since the commonly selected patients for DBI therapy usually include those juvenile and brittle diabetic patients most dependent upon insulin and most easily succumbing to diabetic ketosis. Anorexia, loss of weight, listlessness, and intermittent acetonuria without glycosuria are further indications of overdosage with DBI.

Both metahexamide and chlorpropamide have produced bizarre neurological phenomenon such as asthenia, ataxia, and vertigo in the absence of hypoglycemia. A purported advantage of more prolonged hypoglycemia effects from chlorpropamide has been a disadvantage in my opinion inasmuch as the elderly, senile, arteriosclerotic diabetic patient may suffer irreversible brain injury from such a "beneficial" effect.

No hepatic dysfunction or histological evidence of liver injury has been reported thus far in the 400,000 patients using tolbutamide in the United States. On the other hand, a number of cases of liver injury from the other sulfonylurea compounds has been observed. In fact, I learned that of 1800 patients using metahexamide at least 10 cases of jaundice have been reported. To this I can add 3 more. A 56-year-old man with mild diabetes for 10 years had been managed successfully with 1 gm. of tolbutamide daily for 1 year. A clinical trail of metahexamide, 300 mg. daily, was instituted for approximately 3 weeks; on the twenty-fourth day mild definite jaundice developed with chemical confirmation of obstructive icterus. Liver function studies were otherwise normal, and a biopsy of the liver revealed central lobular necrosis with focal necrosis and mononuclear cell infiltration. This pathological finding resembles the liver injury observed with sulfanilamide in the early days of experience with that drug. In contrast to this type of liver damage I have observed two patients with jaundice due to chlorpropamide in which the picture resembles that of cholestasis. Such a case was a 67-year-old woman who had diabetes mellitus for 15 years, requiring about 100 U. of insulin daily. Three weeks after she had received chlorpropamide, 250 mg. daily, she developed obstructive jaundice which lasted almost 3 months. The biopsy revealed hyperergic cholangiolitis, severe cholestasis (biliary calculi) and focal liver cell injury suggestive of a drug reaction. From other reports it is apparent that a significant number of patients with jaundice of cholestatic type have been and will continue to develop from chlorpropamide therapy while jaundice due to central lobular necrosis will characterize this more serious toxic effect in patients receiving metahexamide. It seems that carbutamide was withdrawn for even less frequent toxicity.

In comparing the side effects and toxicity for the newer oral agents (TABLE 1) with the area of population covered by the therapeutic spectrum of FIGURE 3, it should become apparent that the maximum therapeutic effectiveness with maximum safety is to be found in tolbutamide. If serendipity be responsible for the entire field of oral agents in diabetic treatment, the same happy accident has given us the most widely applicable and safest of these agents in the form of tolbutamide.

Acknowledgment

I am indebted to Hans Papper and Fenton Schaffner for interpretation of the biopsy material.

CORRELATION OF PLASMA LEVELS OF ORAL ANTIDIABETIC AGENTS WITH BLOOD SUGAR RESPONSES

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Medical Hospital of the University of Freiburg, Freiburg i.Br., Germany

With the introduction of sulfonylureas into diabetes therapy 3½ years ago, it could be expected that the substances used in the beginning would not be the most effective. In acute experiments upon human beings as well as upon animals, tolbutamide was found to be more effective than carbutamide. In respect to the considerably longer half-lifetime of carbutamide (on the average, 40 hours[1-3]), however, a more constant plasma level was obtainable with this product than with tolbutamide, which has a half-lifetime of 3 to 8 hours only.[1, 3] Therefore, in long-term therapy carbutamide produces a somewhat better effect. The better effect of chlorpropamide observed by some authors may depend to a great extent on its longer half-lifetime, too (36 to 40 hours [4-6]). According to numerous investigators, therapeutically effective plasma levels of all three products are about the same (10 to 15 mg. per cent).

Occurring for the first time in metahexamide, the relation between plasma level and therapeutic effect seems to be different from that of the sulfonylureas known until the present. The necessary therapeutic doses of metahexamide, as well as its plasma levels, are much lower than those of the other sulfonylurea drugs. In 10 diabetic persons treated with metahexamide, we found plasma levels of 1 to 4 mg. per cent. The lower values were found in the morning; the higher values, at noon time.

For an exact comparison of the different efficacies of tolbutamide and of metahexamide, both substances were given orally and intravenously to healthy fasting adults and blood sugars and sulfonylurea plasma levels were determined.

In addition, some animal experiments were carried out to test whether there is any sign for a different mode of action of the two substances.

Material and Methods

Every 4 to 5 days, 10 healthy, fasting adults were given tolbutamide and metahexamide respectively, in different oral doses (TABLE I) at 8 A.M. under resting conditions. The blood sugars were determined in the capillary blood by the method of Hagedorn and Jensen[7] and by an enzymatic method with glucose oxidase† before application of the medicaments, and 30, 60, 180, 240, and 300 min. thereafter. Venous blood was taken for the determination of the plasma levels, before application of the medicaments, and 1, 4, and 24 hours thereafter. In addition, 10 and 5 healthy persons were given 4 mg./kg. metahexamide and tolbutamide, respectively, intravenously. Blood sugar determinations were carried out in the same manner as for oral application of the medicament. In these cases venous blood was taken for the plasma level determinations before the application of the medicaments, and 3, 30, 60, and 240 min. and 24 hours, thereafter. After oral and intravenous application of 4 mg./kg. metahexamide, urine was collected from 5 persons for a determination of the amount of metahexamide at the same time when blood was taken for the determination of the plasma levels. Finally, 5 persons were given 6 mg./kg. metahexamide intravenously, and the plasma levels of metahexamide were determined 30 min., and 3, 7, 10, and 24 hours thereafter.

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† Supplied by C. F. Boehringer and Sons, Mannheim-Waldhof, Germany.

Tolbutamide plasma levels were determined by the method of Spingler.[8] The determination of the plasma and urine levels of metahexamide was carried out by the method of Bratton and Marshall,[9] in the modification for sodium *p*-aminohippurate by Smith *et al.*,[10] as the original method did not give reproducible results due to the low plasma levels of metahexamide. The recovery of metahexamide was in aqueous solution 96 ± 3 per cent, in plasma 53 ± 1.8 per cent. As in different metahexamide concentrations, the same plasma recovery percentage was found; all measured plasma values were doubled. All plasma level figures were determined by this type of calculation.

TABLE 1

Dosage (mg./kg., orally)	Blood sugar decrease in percentages			
	Metahexamide		Tolbutamide	
	After 60 min.	Maximum	After 60 min.	Maximum
1	12 ± 13.6	14 ± 11.8		
2	16 ± 13.5	28 ± 8.3		
4	24 ± 9.2	30 ± 8.7		
5			1 ± 6.2	15 ± 5.8
10			8 ± 13.0	18 ± 4.2
20			15 ± 5.5	22 ± 3.4
Fasting controls	6 ± 6.8	10 ± 3.8		

(Mean values in 10 healthy adults)

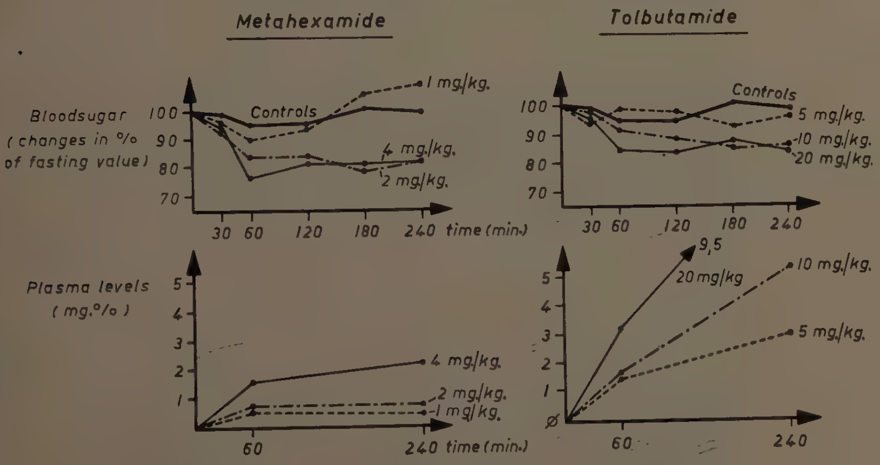
Animal experiments. Eighty albino rats from an inbred stock with a body weight of 250 to 300 gm. were given a standard cake diet; 22 of these animals served as controls. Fifty-eight animals were given 50 mg./kg. metahexamide by an esophagus tube for different periods of time. On 14 rats, blood was taken from the tail vein before the administration of the medicament and 3 hours thereafter. The animals were sacrificed by a blow on the neck 3 and 24 hours, respectively, after a single application of metahexamide, or on the fifth and fifteenth day, respectively, if metahexamide was given daily. The pancreas of each animal was fixed in Bouin's solution, and paraffin sections stained with Azan in the modification of Gomori[11] and with aldehyde-fuchsin phloxin by Maske.[12] From 53 animals a piece of the right median liver lobe was removed immediately after the killing, weighed on the torsion balance, and the liver glycogen determined by the anthrone method of Seifter *et al.*[13]

Results

(1) *Blood sugar decreases, plasma levels, and half-lifetime in healthy adults.* TABLE 1 shows that the maximum blood sugar decrease in the control test was 10 per cent. The oral administration of 1 mg./kg. metahexamide and of 5

mg./kg. tolbutamide did not bring a significant blood sugar decrease. After application of 10 mg./kg. tolbutamide the blood sugar decrease was not very impressive. The application of 2 mg./kg. metahexamide, however, was followed by a blood-sugar decrease of 16 per cent after 1 hour. During a 4-hour period of observation the maximum blood-sugar decrease following this dose was 28 per cent. An adequate effect could be reached only after 20 mg./kg. tolbutamide. Four mg./kg. metahexamide had a better blood sugar decreasing effect than did 20 mg./kg. of tolbutamide.

FIGURE 1 shows, in the upper part, the average values of the blood sugars in the different metahexamide and tolbutamide doses. In the lower part of this figure, the corresponding plasma levels of the two substances are registered. According to a 5-times higher tolbutamide dose, the tolbutamide plasma levels are 5 times higher than in metahexamide. The correlation is not very good in the lower metahexamide doses; it results from the difficulty of an exact determination of plasma levels below 1 mg. per cent.



Mean bloodsugar values and plasma concentrations of metahexamide and of tolbutamide in 10 healthy adults each given three different doses orally.

FIGURE 1.

From these experiments the positive statement can be made that a blood sugar-decreasing effect results from much lower plasma levels of metahexamide than of tolbutamide. Milligram per milligram, metahexamide is at least 5 times more effective than tolbutamide.

FIGURE 2 shows blood sugar and sulfonylurea plasma levels after intravenous application of 5 mg./kg. of metahexamide and tolbutamide, respectively. Tolbutamide given in this dose has no significant blood sugar-decreasing effect; but 30 min. after application of metahexamide the blood-sugar decrease amounts to 35 per cent. The plasma levels of the two substances, however, differ by 20 per cent only. It is difficult to say whether this is due to the method or to a larger distribution space or to a different kind of protein binding. On performing dialysis experiments, we found a firm protein binding of metahexamide. Paper electrophoretic investigations that have not been finished as yet show no self-movement of tolbutamide in sodium diethyl barbituric acid but an elective movement with the albumin, in contrast to metahexamide and carbutamide that show self-movement. Furthermore, on the elution after serum electrophoresis larger amounts of metahexamide and of carbutamide are found in the albumin band.

Figure 3 shows electrophoresis strips of tolbutamide, of carbutamide, and of metahexamide for comparison. At the bottom of each pattern there is a strip with sulfonylurea only, beyond this another one with sulfonylurea in serum, and on the top the same strip as in the middle after protein staining.

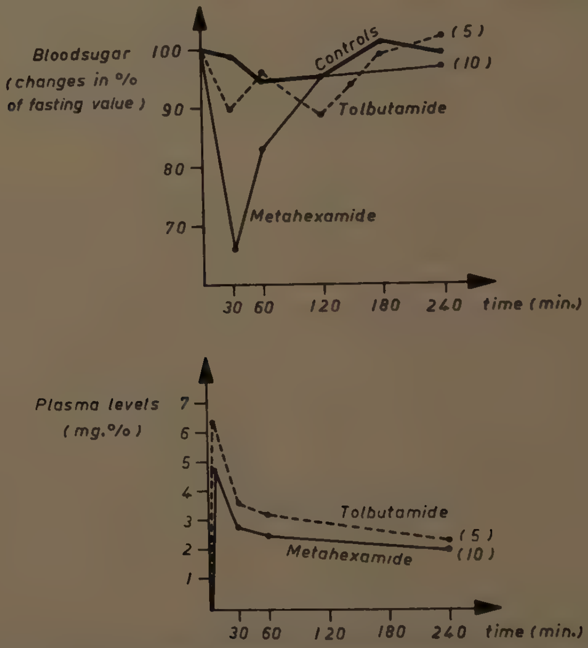


Figure 2. Mean blood sugar values and plasma concentrations of metahexamide and of tolbutamide in 10 and 5 healthy adults respectively, each given 4 mg./kg. intravenously.

Within 24 hours after intravenous application of 280 mg., about 70 per cent of the administered metahexamide was found in the urine (5 persons excreted the following amounts in their 24-hour urines: 207, 242, 191, 199, and 159 mg.). After oral application of 300 mg. of metahexamide 72 per cent was excreted within 24 hours. Within the next 24 hours another 18 per cent was excreted (Table 2). According to these data, after a single oral or intravenous application the half-lifetime of metahexamide is, based on our own observations, only a few hours longer than that of tolbutamide. For this substance we calculated, in correspondence to the literature, a half-lifetime of 5 to 6 hours. The half-lifetimes of metahexamide that we observed with healthy adults were 6 to 16 hours with an average, 7 to 9 hours. The clearance of metahexamide is 10 ml. plasma/min.

(2) *Beta-cell degranulation and liver glycogen in rats after administration of metahexamide.* As metahexamide, according to these results, is considerably more effective than the sulfonylureas used until now, the question may be asked if this substance has the same mode of action as the others. Among other things, after application of sulfonylureas an impressive degranulation of the beta cells in the pancreatic islets[14-17] and an increase of the liver glycogen in fasting animals[18-20] are regarded as constant and typical findings. At present we shall not discuss the extent of the connection between these two phenomena.

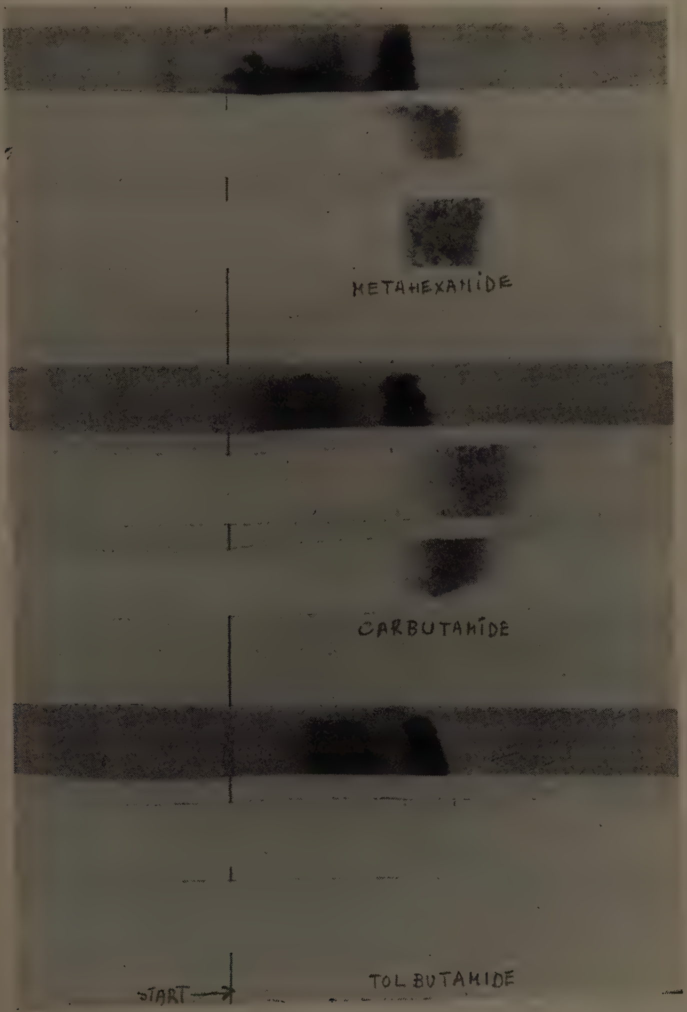


FIGURE 3. Paper electrophoresis of sulfonylureas in a buffer of sodium diethylbarbituric acid. From top to bottom for each of the three substances: (1) serum + sulfonylurea (protein staining with amido black); (2) serum + sulfonylurea (same strip as in (1), staining by the methods of Bratton and Marshall, respectively of Spingler); and (3) sulfonylurea without serum (staining as in 2).

FIGURE 4 shows in the left part that there is a significant increase of the liver glycogen in fasting rats 3 hours after application of metahexamide. At the same time, the blood sugar decrease caused by the dose of 50 mg./kg. is 57 ± 3.2 per cent. Thus far there is no difference between this action and that of the other sulfonylureas. In addition, increased liver-glycogen values had been found after a continued application of tolbutamide if the dose was not higher than 250 mg./kg. body weight.[19,21] This effect is not demonstrable after application of metahexamide, as follows from the right part of FIGURE 4.

TABLE 2

RENAL EXCRETION OF METAHEXAMIDE AFTER A SINGLE ORAL APPLICATION OF 300 MG. (HEALTHY ADULTS)

Case No.	Mg. (0 to 24 hrs)	Mg. (24 to 48 hrs)
1	234	45
2	217	65
3	218	72
4	164	85
5	191	77
6	266	30
7	204	53
8	181	40
9	279	23
Average (mg.)	217	54
Percentages of intake	72	18

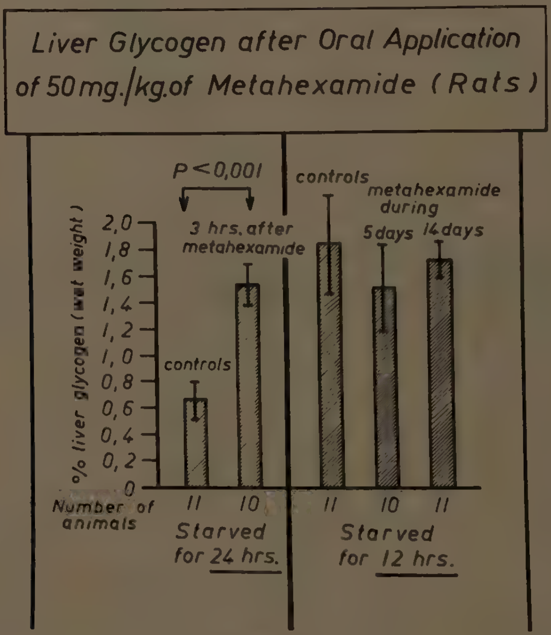


FIGURE 4.

The liver-glycogen values of the animals that were given 50 mg./kg. of metahexamide for 5 and 14 days, respectively, did not differ from the values of the 12-hour-fasting controls.

For comparison, the liver-glycogen contents after application of tolbutamide and of metahexamide have been assessed in TABLE 3. As in our present examinations the liver-glycogen values usually were higher than in the experiments of 3 years ago;[19] the changes have been indicated in percentages of the corresponding control values.

TABLE 3

Variations of liver glycogen in rats

	after	
	Tolbutamide (1956) (250 mg./kg., orally) (percentage)	Metahexamide (1959) (50 mg./kg., orally) (percentage)
3 hrs. after one dose (starved for 24 hours)	+400 ($p < 0.001$)	+230 ($p < 0.001$)
Treated 5 days, last dose 3 hrs. before death (starved for 12 hours)	+250 ($p < 0.02$)	−18 (n.s.)
Treated 14 days, last dose 24 hrs. before death (starved for 12 hours)	+630 ($p < 0.001$)	−7 (n.s.)

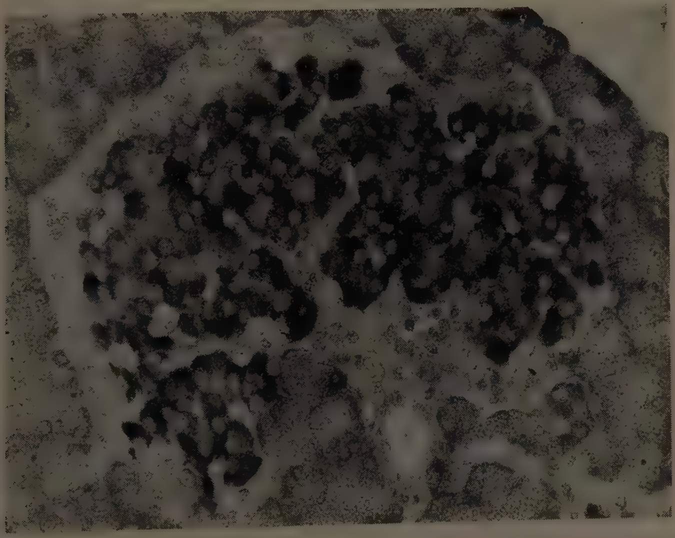


FIGURE 5. Pancreatic islet of a normal rat (fasting control): Bouin's, paraffin section stained with adlehyde-fuchsin phloxin.[15]

We cannot give an explanation for this difference in behavior. Liver toxicity of the substance hardly may be concluded from this, as the liver glycogen did not fall significantly below the values of the control animals.

On the pancreatic islets exactly the same findings were observed after application of metahexamide as after tolbutamide. Three hours after the metahexamide application a beginning degranulation of the beta cells becomes visible. After 24 hours it already is very pronounced. FIGURE 5 shows the normal beta-cell granulation of a pancreatic islet in the rat. FIGURE 6 demonstrates the heavy degranulation of beta cells 24 hours after oral application of 50 mg./kg. metahexamide, and FIGURE 7 the typical picture of completely degranulated beta cells in the pancreatic islets of rats treated for 5 days. After

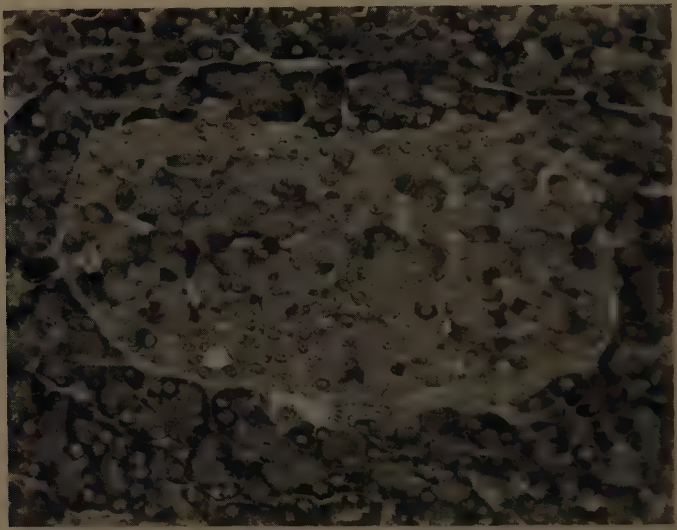


FIGURE 6. Pancreatic islet of a rat, 24 hours after one oral dose of 50 mg./kg. metahexamide. Significant degranulation of the beta cells (stained as in FIGURE 5).

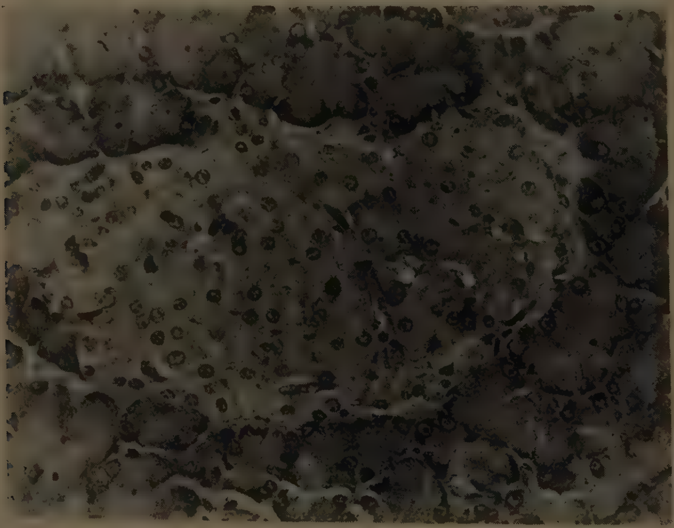


FIGURE 7. Pancreatic islet of a rat after oral application of 50 mg./kg. metahexamide for 5 days. Complete degranulation of the beta cells (stained as in FIGURE 5).

some time there is an adaptation to the treatment with sulfonylureas, so that a moderate regranulation begins despite the continued medication.[14] FIGURE 8 shows a pancreatic islet of a rat after 14 days of uninterrupted metahexamide medication. The beta cells contain some granules again.

In the alpha cells no changes were noticed.

Summary

Our investigations showed that metahexamide is at least 5 times more effective in healthy adults than is tolbutamide. The therapeutically effective plasma level of metahexamide is accordingly only 20 per cent of that needed by tolbutamide. This stronger effect of metahexamide is not due to a protracted excretion, as the half-lifetime is, at the most, twice that of tolbutamide, and considerably shorter than that of carbutamide.

The behavior of the liver glycogen in starved rats and the degranulation of the beta cells after one and repeated applications of metahexamide make it very likely that the mechanism of the blood sugar lowering after metahexamide application is the same as in the other known sulfonylureas. If, therefore, the stronger effect of metahexamide means a remarkable progress, there remains for elucidation only the problem of whether this substance is as nontoxic as tolbutamide.

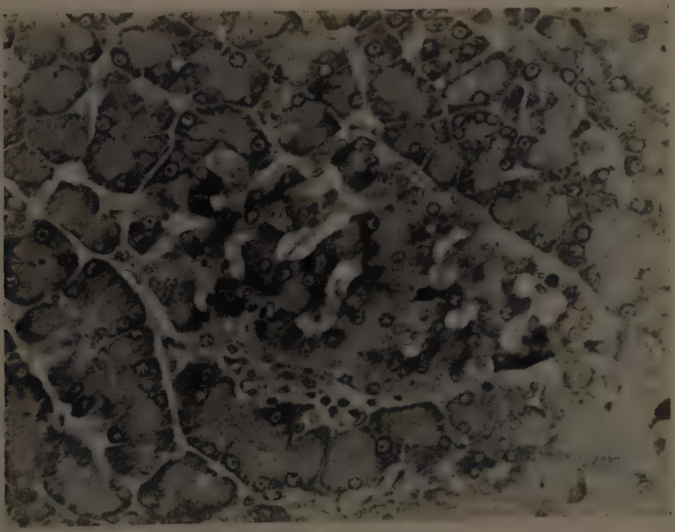


FIGURE 8. Pancreatic islet of a rat after oral application of 50 mg./kg. metahexamide for 14 days. Slight regranulation of the beta cells (stained as in FIGURE 5).

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COMPARATIVE PHARMACOLOGY AND CLINICAL RESPONSES TO METAHEXAMIDE*

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Metahexamide (N-(3 amino-4-methyl benzine-sulfonyl)-N^1-cyclohexylurea) was studied for the purpose of comparing its hypoglycemic potency, toxicity, and pharmacology with the currently existing compounds used for oral therapy of diabetes.

Observations made during a 6-month period in which metahexamide was given in daily doses ranging from 50 to 500 mg./day to 73 maturity-onset diabetic patients permit the conclusion that this compound embodies a hypoglycemic potency that is approximately 7 times that of tolbutamide and twice that of chlorpropamide. In addition, normoglycemia is obtained in responsive patients with serum concentrations of metahexamide that range from one fifth to one third as high as the levels needed with the other sulfonylureas. Early studies of the incidence of untoward reactions to metahexamide indicate an incidence of about 10 per cent. This incidence is comparable to that of chlorpropamide. However, preliminary data suggest that alterations of liver function are more frequent with metahexamide than with other compounds.

METHODS

Seventy-three diabetic patients were selected for treatment with metahexamide during the 6-month period between September 1958 and March 1959. Individuals who had been treated previously with chlorpropamide and tolbutamide were chosen deliberately. All patients were maturity-onset diabetics who repeatedly demonstrated fasting and postprandial blood glucose values exceeding 150 mg. per cent in spite of appropriate diet therapy. Metahexamide was given once daily in doses ranging from 50 to 500 mg. for periods of 15 to 180 days. Insulin was discontinued at the onset of therapy in patients who were using it, and in all cases the usual diet was continued. In the patients who had been managed with chlorpropamide, metahexamide was substituted in the same daily dosage where feasible, and the average blood glucose levels obtained during therapy with each agent were compared. Patients who consistently maintained fasting and postprandial blood glucose levels below 150 mg. per cent were regarded as responsive while those displaying values above this critical level were considered unresponsive. Control values of liver function, renal function, and hematopoietic function were established by measurement of cephalin flocculation, serum bilirubin, thymol turbidity, albumin, globulin, cholesterol, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGO-T), Bromsulphalein (BSP) retention, hematocrit, white cell counts, differential counts, prothrombin times, blood urea nitrogen (BUN), creatinine, and uric acid concentrations. Other studies included protein-bound iodine, thyroidal radioactive iodine uptake, and urinary excretion of 17-ketosteroids and 17-hydroxycorticoids. In most patients blood glucose and serum metahexamide levels[1, 2] were obtained at weekly intervals, and the other indices were obtained frequently during the period of therapy with metahexamide.

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* The work reported in this paper was supported in part by The Comly Coleman Foundation of The Ohio State University; Training Grant Nos. 2A-5118(C) and 2A-5118(C) from the United States Department of Health, Education, and Welfare, Washington, D. C.; The Upjohn Company, Kalamazoo, Mich.; Eli Lilly and Company, Indianapolis, Ind.; Chas. Pfizer & Co., Inc., Brooklyn, N. Y.; and U. S. Vitamin Corporation, New York, N. Y.

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Results and Discussion

Pharmacological Studies

Dosage. Favorable hypoglycemic effects were seen with daily doses ranging from as low as 50 mg./day to as high as 250 mg./day. The average dose of metahexamide on the day that normoglycemic levels were achieved in the first 30 responsive patients was 235 mg./day. The dose needed to preserve such levels decreased following response so that the average current maintainance dose is 150 mg./day. Figure 1 compares the ranges of daily maintenance dosage of metahexamide with chlorpropamide in responsive patients. In the 42 patients responsive to metahexamide it was necessary to employ 5 different daily dose levels. It is interesting to note than an approximately equal number of patients fell into each dose group ranging from 50 to 250 mg./day. The mean maintenance dose for the entire group was 149 mg./day. In contrast, 77 per cent of the 62 chlorpropamide-responsive patients required daily maintenance doses of 200 mg. or more and averaged 282 mg./day, which is approximately twice the amount needed with metahexamide. A similar decrease in maintenance requirement with chlorpropamide was observed as the dosage fell from 435 mg./day at the time of initial response to 282 mg./day. This tend-

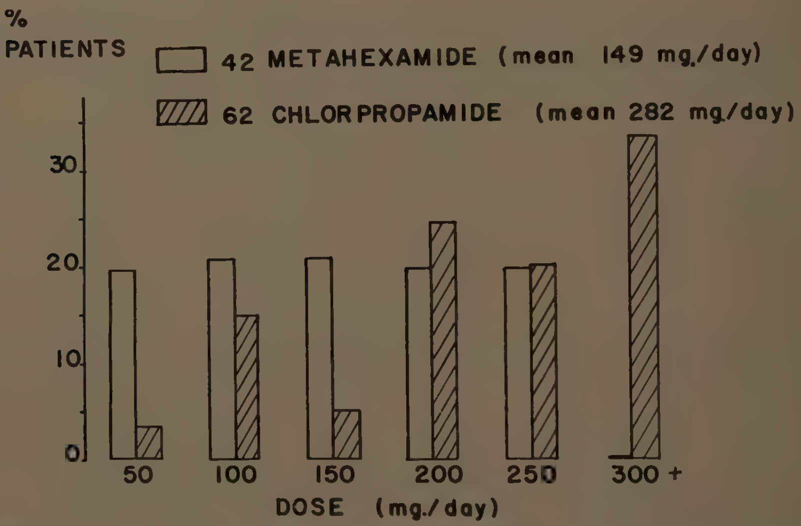

Figure 1. Daily maintenance doses of metahexamide with chlorpropamide in responsive patients.

ency for the magnitude of dosage to decrease during maintenance is of clinical importance as these potent hypoglycemic agents may produce serious hypoglycemia if patients are not observed closely and the dosages carefully regulated.

Regardless of the dosages used, serum levels of metahexamide were found to be lower than those found with comparable doses of the other sulfonylureas. Striking variations of levels were found in individuals receiving the same daily doses of metahexamide, but in general a rough correlation between the daily dose and the serum levels was noted (FIGURE 2). For example, patients taking 150 mg. daily (a value subsequently found to be the dosage mode) averaged 3.0, and ranged from 1.1 to 5.3 mg. per cent. Serum levels tended to plateau when larger doses were employed so that dosages as high as 500 mg. daily resulted in a mean serum level of only 6.16 mg. per cent. The latter value contrasts sharply with the mean level of 20 mg. per cent attained utilizing a 500-mg. dose of chlorpropamide in the same patients. The contrast between effective serum levels of the 2 compounds is presented in FIGURE 3. It may also be noted that maximum serum levels are achieved only after 6 to 8 days of administration of a constant daily dose. The observed increment in drug concentration during initial therapy is probably best explained by the relatively

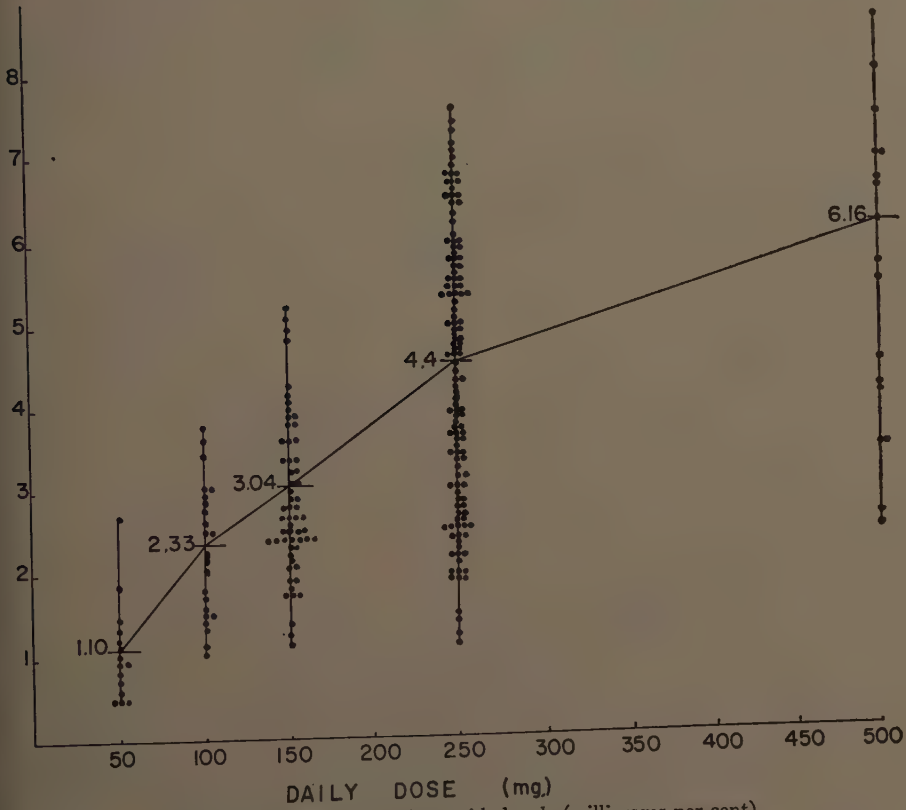

FIGURE 2. Plasma metahexamide levels (milligrams per cent).

long half lives of these compounds. It has been shown previously that the biological half life for chlorpropamide averages 34 hours.[3, 4] Our studies indicate that serum concentrations of metahexamide fell 50 per cent within 17.5 to 22 hours (average 19 hours) following the oral ingestion of this compound (FIGURE 4). It is of further interest that, in the 4 individuals in whom the half-life study was done, calculations of a theoretical "metahexamide space" averaged 12 l., and in no patient was this thought to be in excess of extracellular fluid volume.

To demonstrate the differences in serum levels attained in the same patient on the same dosage of these 2 compounds, a group of 15 responsive patients was followed for 2 months. During the first month some received metahexamide and the others received chlorpropamide. During the next month the drugs were alternated, so that each patient who had received metahexamide was placed on chlorpropamide in the same dose, and vice versa. FIGURE 5 demonstrates that on the same dose the metahexamide serum levels are distinctly lower than the serum levels obtained on chlorpropamide. The explanation for this difference in serum levels of these 2 compounds is still doubtful. It is clear, however, that the effective serum levels of metahexamide are one fifth to one half of the serum levels obtained with chlorpropamide.

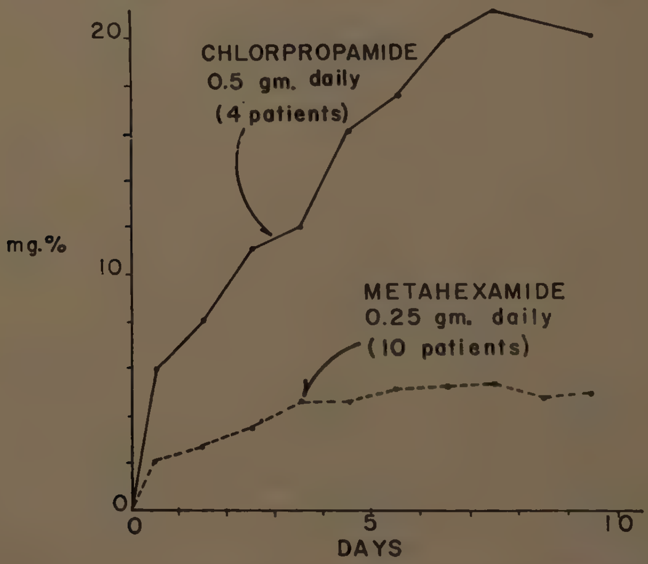

FIGURE 3. Blood levels achieved with maximum daily maintenance doses.

Hypoglycemic effect. Forty-four of the 73 patients (60 per cent) responded to metahexamide in a satisfactory fashion. In each instance blood glucose values fell from hyperglycemic levels to fasting and postprandial levels which remained below 150 mg. per cent during the entire period of therapy. The mean daily maintenance dose of metahexamide in the responsive group was 150 mg. and ranged from 50 to 250 mg. This dose resulted in a mean serum level of 3.9 mg. per cent, but considerable variations in serum concentrations ranging from 1.4 to 5.8 mg. per cent were observed. Some of the characteristics of the responsive and nonresponsive patients are listed in TABLE 1. It may be noted that the responsive patients as a group had been taking less insulin and reported a shorter duration of diabetes than did the nonresponsive patients.

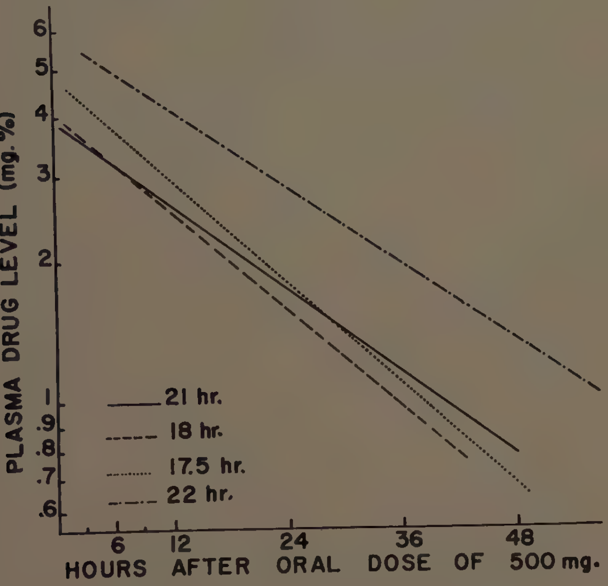

FIGURE 4. Plasma half life of metahexamide.

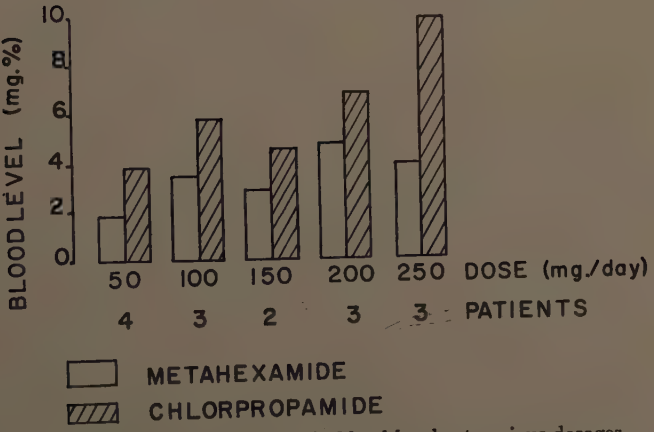

FIGURE 5. Differences in blood levels at various dosages.

Response to metahexamide was often delayed so that after seven days of therapy regardless of the dose or the serum metahexamide level, 40 per cent of patients who ultimately reached normoglycemic levels had not yet responded. This characteristic delay in response is graphically presented in FIGURE 6 and has been observed with the other sulfonylureas.[4]

Standard oral glucose tolerance tests were performed before and after 1 week of metahexamide therapy in 8 responsive patients. As demonstrated in FIGURE 7, no essential change in the configuration of the curve occurred.

TABLE 1
CHARACTERISTICS OF PATIENTS*

Factor	Responsive (44)	Nonresponsive (29)
Age (years)	61	61
Per cent, women	55	83
Daily insulin (U.)	11	22
Years of diabetes	4.6	7
History of acidosis (%)	0	14.0
Dose (mg./day)	150	250
Mean blood level	3.04	5.48
Per cent fall in blood sugar	53.0	13.7

* Figures given as mean.

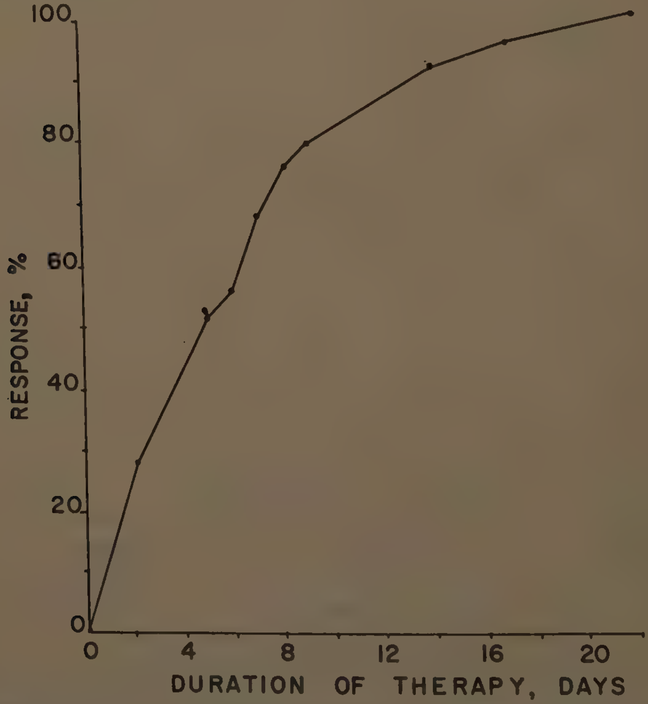

FIGURE 6. Frequency of delay in response to metahexamide in 25 patients.

Although the mechanism of this effect is not clear, this is not the response that one would predict from the administration of insulin.

Comparative hypoglycemic effect. There were 53 instances in which the response to metahexamide could be compared to that with tolbutamide and chlorpropamide in the same patients. Metahexamide was given to 20 individuals who previously had been treated with tolbutamide. Seventeen of these subjects were deliberately selected because they had been shown to be unresponsive to tolbutamide. Six of the 17 were found to respond to metahexamide. All of the tolbutamide-responsive patients responded to metahexamide. This finding indicates that metahexamide may prove useful in tolbutamide-resistant patients.

Comparative observations were made in 33 patients who had undergone trials with both chlorpropamide and metahexamide. On the basis of response, these patients could be divided into 3 groups (TABLE 2).

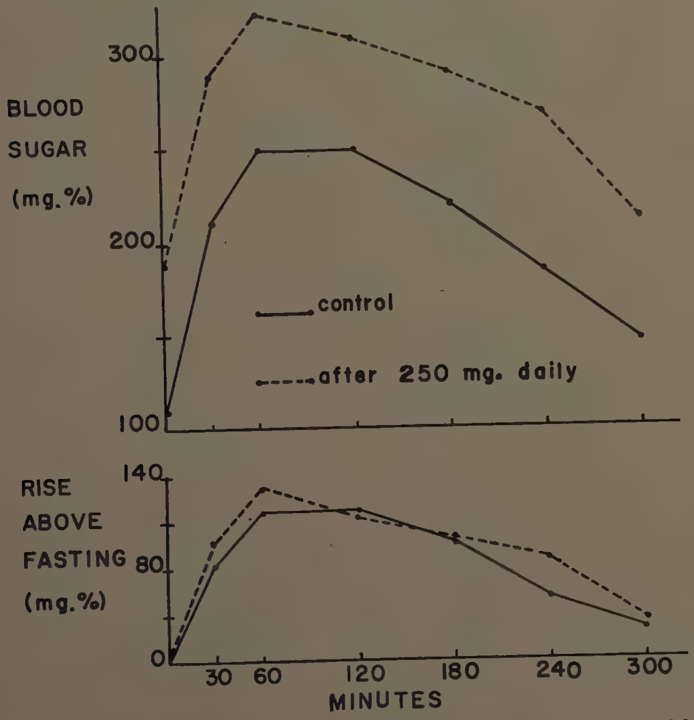

FIGURE 7. The influence of metahexamide on oral glucose tolerance (means of 8 patients).

Seventeen individuals (51 per cent) were responsive to both compounds (Group 1), and 3 patients (9 per cent) proved sensitive to chlorpropamide but not metahexamide, Group 2. Thirteen individuals (40 per cent) failed to respond to either compound. No patient was found to be responsive to metahexamide but resistant to chlorpropamide. From these observations one might postulate that chlorpropamide may occasionally be effective in the metahexamide-resistant patient. It may be of interest to note that in 5 of the 13 subjects found to be unresponsive to all of the sulfonylureas, Phenformin* proved to be an effective hypoglycemic agent, although its use was curtailed by gastrointestinal intolerance in 2 patients.

* Product of U.S. Vitamin and Pharmaceutical Corporation, New York, N. Y.

TABLE 2
COMPARISON OF DOSAGE AND BLOOD SUGAR LEVELS IN 33 DIABETIC PATIENTS TRANSFERRED FROM CHLORPROPAMIDE TO METAHEXAMIDE

	Chlorpropamide trial period			Metahexamide trial period		
	FBS (mg.%)*	Daily dose chlorpropamide (gm.)*	Serum level (mg.%)*	FBS (mg.%)*	Daily dose metahexamide (gm.)*	Serum level (mg.%)*
Group 1: 17 patients responding optimally to both agents	113 (80 to 150)	0.287 (0.05 to 1.0)	11.1 (3.5 to 23.0)	97.8 (70 to 150)	0.176 (0.05 to 0.50)	3.4 (1.0 to 6.9)
Group 2: 3 patients responding optimally to chlorpropamide only	135 (110 to 150)	0.267 (0.10 to 0.50)	12.1 (5.6 to 23.0)	213 (180 to 240)	0.167 (0.10 to 0.25)	3.8 (2.9 to 5.4)
Group 3: 13 patients responding to neither agent	248 (180 to 400)	0.438 (0.20 to 1.0)	16.9 (8.5 to 28.0)	237 (165 to 300)	0.254 (0.15 to 0.40)	5.5 (3.1 to 9.8)

* Figures given as mean and range.

Other Effects

Endocrine effects. Studies of patients with acute and chronic conditions suggest that metahexamide has no important effect on thyroid or adrenal function.

TABLE 3
ENDOCRINE FUNCTION STUDIES

	Test	Control*	Therapy*	Days interval*	No. patients
Thyroid	24-hr. I^{131} uptake (17 to 37%)	20 (11 to 37)	18 (7 to 33)	56 (7 to 105)	25
	Serum PBI (3.5 to 8 µg.%)	5.1 (2.95 to 8.72)	5.2 (2.9 to 7.78)	64 (7 to 120)	34
	Serum cholesterol (130 to 280 mg.%)	238 (113 to 400)	225 (150 to 395)	64 (7 to 120)	43
Adrenal	17 OH/24 hr. (5 to 15 mg.)	8.6 (0.5 to 23.8)	12.4 (1.1 to 14.0)	5 (1 to 9)	12
	17 ketosteroids/24 hr. (5 to 20)	6.26 (5.6 to 30.1)	6.65 (1.1 to 14.8)	5 (1 to 8)	11

* Mean and range.

TABLE 4
HEMATAPOIETIC AND RENAL FUNCTION TESTS

Tests	Control*	Therapy*	No. patients	Days interval*
Hb	13.2 gm. % (10.5 to 15.9)	12.4 (8.0 to 15.8)	41	71 (7 to 135)
Hct.	42.5% (35 to 51%)	40.0% (30 to 53%)	29	71 (7 to 135)
WBC	8,382/mm.^{3} (3500 to 22,400)	7,086 (4500 to 12,850)	54	63 (7 to 120)
Platelet count	213,124/mm^3 (34,000 to 728,000)	214,933 (116,000 to 424,000)	14	10 (6 to 14)
Bleeding time	1′23″ (45″–2′0″)	2′5″ (55″ to 6′0″)	9	73 (4 to 150)
Clotting time (cap.)	6′0″ (3′25″ to 7′35″)	3′10″ (1′4″ to 7′15″)	7	73 (4 to 150)
BUN	18 mg.% (9 to 44)	18 (10 to 45)	48	71 (7 to 135)
Creatinine	1.6 mg.% (1.0 to 2.7)	1.8 (1.0 to 3.4)	14	71 (7 to 135)
Uric acid	3.5 mg.% (2.7 to 5.9)	3.2 (0.8 to 7.4)	13	71 (7 to 135)
PSP	15 min. 30% 2 hr. 67%	15 min. 20.25% 2 hr. 43.75%	2 2	9 (7 to 10)

* Mean and range in each instance.

Values of these indices of endocrine function obtained before and during treatment are presented in TABLE 3.

Effects on hematopoietic and renal function. No changes in renal function as reflected by alterations of BUN, creatinine, PSP excretion, or changes in urinary sediment were noted. These findings are presented in TABLE 4. A single instance of hemolytic anemia believed to be induced by metahexamide was observed and is discussed below.

Effects on liver function. Values pertaining to several liver functions were determined over periods of observation ranging from 14 to 180 days (TABLE 5). Sporadic elevations of thymol turbidity (2 patients) serum alkaline phosphatase (2 patients), and SGO-T (1 patient), usually of minor proportion, were observed. In these patients such deviations were transient and were seen in patients with a definite history of alcoholism or cholelithiasis. In one instance, a causal relationship of derangement of liver function seemed clearly related to metahexamide therapy.

This patient was a 46-year-old Negro woman whose case has been reported in detail elsewhere.[6] She had been diabetic for 7 years and, after having been treated with metahexamide 250 mg. daily for 15 days, she was admitted to the hospital with jaundice and diabetic acidosis. Laboratory studies on admission showed chemical evidence of liver damage. The total serum bilirubin rose from normal values to 16 mg. per cent on the thirtieth day; the alkaline phosphatase rose from 3.5 to 36.8 Shinowara, Jones, and Reinhart (SJR) units and the SGO-T rose to 263 units. During the period of jaundice the hemoglobin fell from 11.8 gm. per cent to 7.3 gm. per cent and the reticulocyte count rose to as high as 9.2 per cent. A study of RBC survival using Cr^{51}-tagged cells indicated a slight decrease in RBC survival time. Four serial punch biopsies of the liver showed transient changes characterized by mild focal hepatocellular necrosis, minimal periportal round cell infiltration, and inspissation of bile in the biliary canaliculi. The red blood cell content of glucose-6-phosphate dehydrogenase was found to be reduced. These findings are interpreted as consistent with metahexamide-induced cholestasis with jaundice and metahexamide-induced hemolytic anemia in a patient with a congenital deficiency in glucose-6-phosphate dehydrogenase. Continued observation of this patient during the 3 months since the onset of her jaundice indicates that there is no residual evidence of hepatic damage.

Side reactions. Untoward effects believed attributable to metahexamide were observed in 7 of the 73 patients (10 per cent).

Four individuals developed epigastric distress that occurred after ingestion of the tablets. Each of these patients had complained of similar distress prior to trial on the experimental drug, but noted relief from this complaint within a few days after therapy with metahexamide was interrupted. X-ray studies of the upper gastrointestinal tract in 2 of these patients showed no evidence of ulcer, gastritis, or abnormal peristalsis.

One patient developed a generalized maculopapular skin eruption on the thirty-first day of therapy on a 250 mg./day dose. The rash was quite extensive but cleared completely within 7 days after the drug was discontinued. A second patient developed rather severe generalized pruritis that was not associated with an eruption. This symptom also regressed promptly after therapy was terminated.

Although variations in liver function studies occurred in 6 patients, in only 1 instance could such changes be clearly related to metahexamide administration; this instance of jaundice with hemolytic anemia is described above. It might

TABLE 5
LIVER FUNCTION TESTS

Tests	Control*	Therapy*	No. patients	Days interval*
TP A/G	7.6 3.4/3.3 mg.% (6.2 to 8.8) (3.7 to 5.4)/(2.6 to 4.1)	7.8 4.2/3.1 (6.4 to 8.4) (2.7 to 4.9)/(2.2 to 4.3)	29	79 (7 to 150)
Transaminase	14.7 units (4 to 21)	22.5 (13 to 46)	10	69 (3 to 135)
Bilirubin	T 0.45 mg.% (T 0.1 to 0.8)	T 0.3 (T 0.1 to 0.4)	14	71 (7 to 135)
Cephalin floc.	neg. (neg. to 3+)	neg. (neg. to 2+)		82 (14 to 150)
Thymol turb.	10 units (neg. −20)	10 (neg. −30)	37	79 (7 to 150)
Alk. phos.	5.8 SJR units (3.6 to 9.2)	6.4 (2.6 to 20.7)	35	70 (4 to 135)
BSP	7% (0 to 21%)	4.5% (2 to 11)	4	7 (4 to 10)
Prothrombin time	78% (50.5 to 100%)	76.8% (45.1 to 100)	21	70 (5 to 135)

* Mean and range in each instance.

be postulated that in some instances metahexamide may induce changes in liver function that are transient and do not necessarily indicate important toxic manifestations. It will be necessary to observe a large number of patients treated for chronic conditions in order to be able to interpret the significance of such transient elevations of serum alkaline phosphatase, thymol turbidity, or SGO-T, particularly in a group of patients with a potentially high incidence of nondrug-related liver damage.

Summary and Conclusions

(1) Metahexamide was found to effect normoglycemia in 44 of 73 maturity-onset diabetics who previously could not be controlled with diet.

(2) The daily dose of metahexamide needed for response varied between 50 and 250 mg. and averaged 150 mg., which is approximately one half and one seventh of the average doses required with chlorpropamide and tolbutamide, respectively.

(3) Normoglycemia was achieved with metahexamide in responsive patients with blood levels ranging from 1.4 to 5.8 mg., and averaged 3.9 mg. per cent. This level is 20 to 33 per cent as high as levels reported as needed with all other sulfonylureas.

(4) The biological half life of metahexamide averaged 19 hours as compared with the average half lives of 34 hours and 4 hours reported for chlorpropamide and tolbutamide, respectively.

(5) Although serum levels within the therapeutic range may be achieved within 4 hours, normoglycemia still had not been achieved after 7 days in 40 per cent of the patients who ultimately responded.

(6) Metahexamide was effective in 6 of 17 tolbutamide-resistant patients and in all of 3 tolbutamide-responsive patients. Metahexamide was effective in 17 of 20 patients who were responsive to chlorpropamide. In no patient resistant to chlorpropamide was a favorable response obtained with metahexamide.

(7) This compound produced no changes in thyroid, adrenal, cardiac, or renal function.

(8) In a total of 73 patients exposed to metahexamide there were 4 instances of gastrointestinal intolerance, 1 instance of generalized maculopapular eruption of the skin, 1 instance of generalized pruritis without eruption, and 1 instance of combined toxic hepatitis and hemolytic anemia. In addition, there were 5 instances of sporadic transient abnormalities in liver function tests, the significance of which is obscure.

(9) It is felt that tolbutamide remains the drug of choice for initiation of oral hypoglycemic therapy. Metahexamide and chlorpropamide, because of their enhanced potency, are frequently useful in patients found resistant to tolbutamide. Chlorpropamide may be effective in an occasional metahexamide-resistant patient.

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TOLBUTAMIDE STUDIES IN PREDIABETES*

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INTRODUCTION

It has been shown that the oral hypoglycemic agents of the sulfonylurea type produce hypoglycemia by a mechanism that requires the presence of the beta cells of the pancreas.[1] Evidence of many types has accumulated confirming this observation, indicating that insulin is released from the pancreas as a result of the action of these drugs. The evidence for insulin release includes the following: (1) cross-circulation experiments showing the hypoglycemic effect of pancreatic venous blood;[2] (2) histological demonstrations of alterations in the granulation of beta cells;[3] (3) comparative studies of the metabolic changes following insulin and sulfonylurea hypoglycemia; and (4) clinical data relating responsiveness to the drugs to the adult rather than the juvenile onset type of diabetes.

If, indeed, this is the mechanism of action of these agents, it seems reasonable to conclude that the magnitude and type of blood-sugar response to these agents must be dependent upon the quantity of *available* insulin in the pancreas. In this connection, Unger and Madison recently published observations of 75 mild diabetics and 100 normal subjects studied after the administration of 1.0 gm. of tolbutamide intravenously.[4] A striking difference was noted in the response of the diabetic. Not only was the 30-min. fall in blood sugar reduced, namely 82 per cent of cases showed a blood sugar fall of less than 23 per cent of the initial blood sugar as contrasted with 99 per cent of the normals who showed a fall greater than 23 per cent, but the shape of the curve was quite different. The diabetic response was characterized by a gradual but continuously falling blood-sugar curve even at 90 min., while the normal response was a rapid fall within 30 min. and a rapid return toward the starting value (FIGURE 1).

It is not surprising that in the diabetic the decline in blood sugar is of smaller magnitude. The continuous fall in sugar, on the other hand, suggests that in the diabetic, differences may exist that relate not only to the insulin content of the pancreas but also to the rate of insulin release. In an effort to gain some understanding of the nature of the normal and diabetic curve, studies were made of the tolbutamide response of normal dogs and normal human subjects to intravenous tolbutamide. The latter was investigated under conditions expected to influence the state of carbohydrate tolerance. During the course of these studies it was noted that certain subjects could be converted from a normal response to that entirely characteristic of the diabetic. These subjects, using the terminology of Conn,[5] have been designated as prediabetics.

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* The work reported in this paper was supported in part by grants from The Upjohn Company, Kalamazoo, Mich., and Merck Sharp & Dohme, West Point, Pa.

† Rockefeller Foundation Fellow.

MATERIALS AND METHODS

Animal Experiments

Experiments were performed upon 7 mongrel dogs weighing between 15 and 20 kg. each. Animals were maintained on routine laboratory diets and fasted overnight prior to study. The experimental procedure was performed under Pentothal anesthesia. The abdomen was opened and splenectomy was performed. Following this, a No. 60 polyethylene catheter was inserted retrograde into the pancreatic vein. Blood was collected in heparinized tubes at approximately 5 cc./min. A control sample was taken after 15 min. of rest. Ten minutes later the true zero-time sample was collected. Following this, tolbutamide, 60 mg./kg., was rapidly injected into a peripheral vein. Thereafter pancreatic venous blood samples were collected from the catheter every 10 min. over a 30-min. period. Peripheral venous samples were obtained at similar intervals. Blood sugars were determined by the Somogyi-Nelson procedure.[6] Pancreatic venous samples were centrifuged in the cold. The plasma was then diluted 1:6 in Ringer-phosphate buffer, pH 7.4. The final glucose concentration was made up to 200 mg. per cent. An assay for insulin activity was performed on these samples using the paired-rat hemidiaphragm technique in a modification of the method of Randle.[7] In each determination, hemidiaphragms obtained from 3 rats were pooled, weighed, and placed in paired Dubnoff flasks. One flask contained 2 ml. of the control plasma-buffer

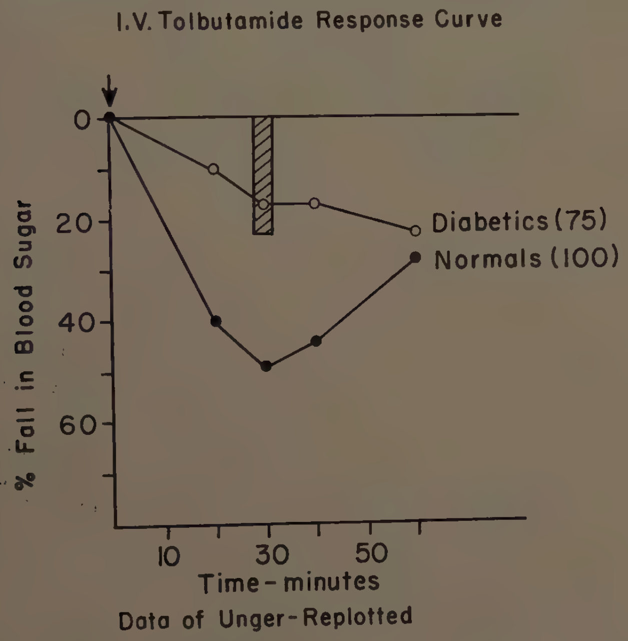

FIGURE 1. Composite curves showing the normal and diabetic blood sugar response to tolbutamide.[4]

solution, while the second flask contained the 10-, 20-, or 30-min. samples. Plasma samples were run in triplicate. In this way data could be expressed as the change in glucose uptake of the diaphragm incubated in the 10, 20, and 30-min. samples following tolbutamide as compared with the 0-min. sample. Insulin activity was then expressed as the change in micrograms of glucose taken up per 100 mg. diaphragm per hour. These values were not calculated back to the undiluted plasma. The pancreatic samples were also tested for hyperglycemic activity. Plasma samples were diluted 1:3 in Ringer-phosphate solution without added glucose. Liver slices weighing approximately 200 mg. were obtained from fed rats and were incubated in 2 ml. of diluted plasma at 37° C. in the presence of oxygen for 1 hour. At the completion of the experiment, measurements of glucose appearing in the medium were made. The glucose release (μg. glucose/100 mg. liver/hour) of the zero time sample was compared with quadruplicate samples obtained at 10, 20 and 30 min. after tolbutamide; the activity was expressed as this change. In a few instances peripheral samples of plasma were assayed for both insulin and hyperglycemic activity.

Human Experiments

A group of 28 normal subjects consisting of 7 females and 21 males were studied. These subjects were young laboratory workers and medical students. The group was selected in such a way that 17 individuals had a known family history of diabetes. All subjects were on standard diets and were fasted overnight prior to testing. The blood sugar response to 1 gm. sodium tolbutamide administered intravenously in a 2-min. period was then studied under varying conditions. Blood samples were obtained every 15 min. for 1 hour and then at 90 min. Sugars were done in duplicate. The nonesterified fatty acids were measured by the method of Dole.[8] Steroids, when used, were Decadron in total dosage of 6 mg. taken orally 8½ and 2 hours prior to testing and, in a few cases, 40 mg. of Prednisone was used.

RESULTS

Animal Experiments

Insulin activity following I.V. tolbutamide. The mean value of glucose uptake by diaphragm incubated in zero-time pancreatic plasma was 320 μg. glucose/100 mg. wet diaphragm/hour. The mean changes observed in pancreatic plasma compared with the zero-time plasma value in the 7 dogs tested may be seen in FIGURE 2. Correlation with the changes in blood sugar also may be seen. It may be noted that the maximal change was an increment in the 10 to 20 min. sample following tolbutamide, with a fall noted thereafter. These changes are significant by analysis of paired data ($p = 0.01$). No change was noted in peripheral blood, using the zero time peripheral sample as control.

Hyperglycemic activity following I.V. tolbutamide. In five dogs studied (FIGURE 3), there was a decrease in the release of glucose from rat-liver slices incubated in pancreatic venous samples following tolbutamide. No comparable changes were noted in peripheral blood. Since glucagon-free insulin tested *in vitro*, using zero-time pancreatic plasma as a vehicle had no effect on glucose release from liver slices, it seemed likely that this effect of pancreatic plasma following tolbutamide was not due to the increased insulin activity but to a possible decrease in glucagon occurring at the time of insulin release. It should be noted that this decrease in glucose release was of the order of 300 μg. glucose/100 mg. liver/hour, while the initial glucose release was 2.0 mg./100 mg./hour. Despite considerable variation, the observations were consistent.

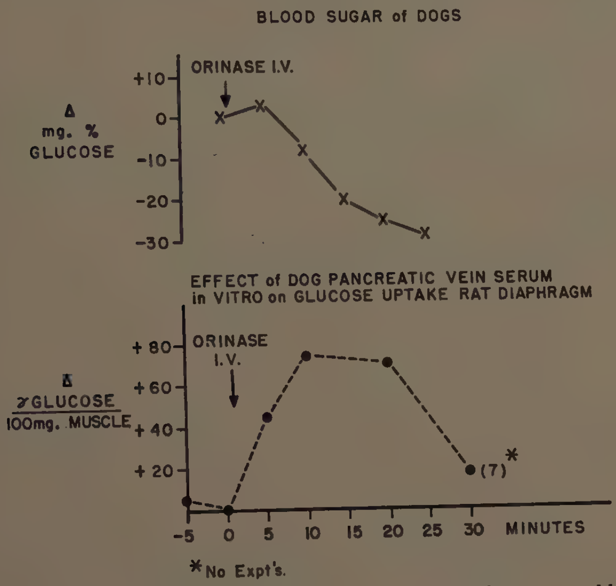

FIGURE 2. Assay for insulinlike activity in dog pancreatic venous blood following tolbutamide.

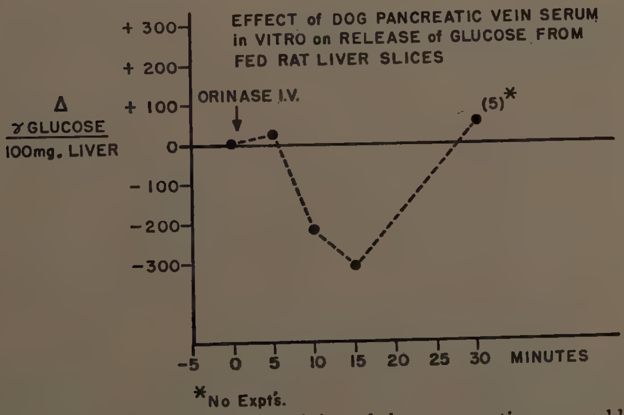

FIGURE 3. Assay for hyperglycemic activity of dog pancreatic venous blood following tolbutamide.

These observations in the dog demonstrate that following I.V. tolbutamide, there is a rapid increase in insulin activity in pancreatic vein blood. This activity does not appear to be sustained, although observations were not made over longer periods than 30 min. In addition, as insulin is secreted, there appears to be a decrease in secretion of hyperglycemic factor.

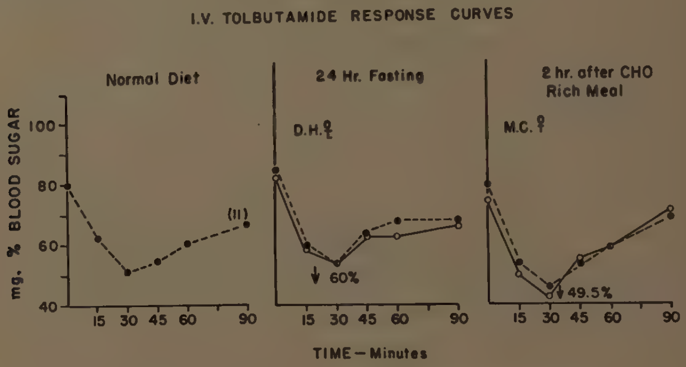

FIGURE 4. Influence of diet on tolbutamide response.

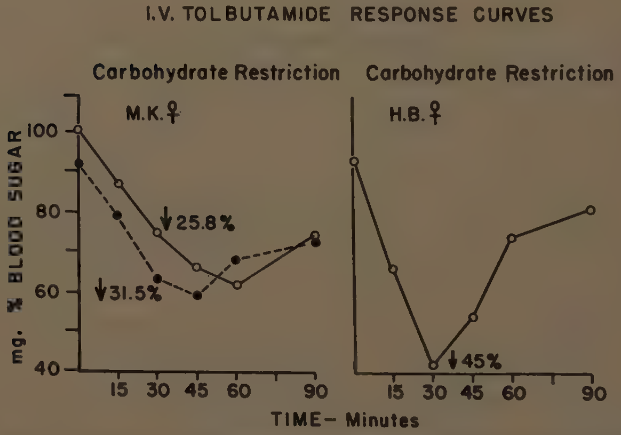

FIGURE 5. Influence of carbohydrate deprivation on tolbutamide response.

Human Experiments

Effect of diet on I.V. tolbutamide response. In 11 normal subjects on a standard diet containing liberal amounts of carbohydrate, the blood sugar response to tolbutamide was similar to that described by Unger (FIGURE 4). Using one of his criteria for differentiating normal and diabetic curves it may be noted that all but 1 subject, at 30 min., showed a fall in blood sugar greater than 23 per cent of the initial value. In addition, the maximal fall in blood sugar tended to occur at 30 min. although in a few instances it was at 45 min. These criteria appear to be characteristic of the normal response to tolbutamide.

FIGURE 4 also shows the effect of a 24-hour period of starvation in a subject previously tested while on a normal diet. It is of interest to note that no change in the curve can be seen. Similarly, performance of the test after 24 hours of excessive carbohydrate intake and 2 hours after a high carbohydrate meal produced no alteration in the response. However, in 2 subjects in whom carbohydrate was eliminated for a period of 3 to 7 days and who were maintained on isocaloric diets, high in protein and fat, 2 different responses were noted (FIGURE 5). Subject H.B. showed a response which was entirely normal. Carbohydrate deprivation produced a change in response in subject M.K., who, incidentally, has a very extensive family history of diabetes. Prior to carbohydrate deprivation, the time of maximal blood sugar fall following tolbutamide was 45 min. with a fall at 30 min. of 31.5 per cent. Following deprivation, the time of maximal fall was 60 min., and the fall at 30 minutes was 25.5 per cent. This delay in time of maximal fall in blood sugar following carbohydrate restriction, approaches the diabetic curve in which the time of maximal fall is delayed beyond 90 min.

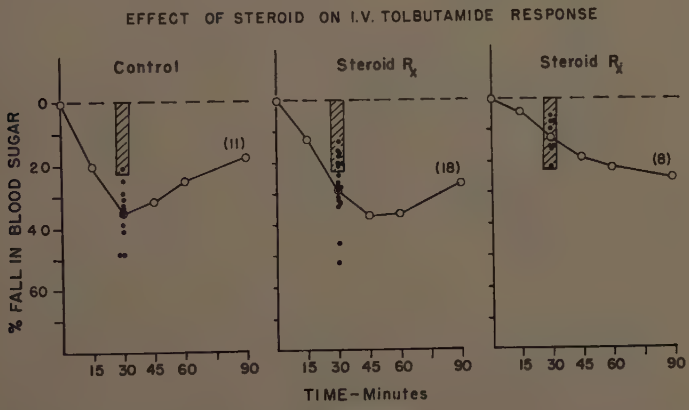

FIGURE 6. Composite curves with the spread of the 30-min. values indicated. The bar indicates the 23 per cent level of blood sugar fall.

Effect of Steroids on I.V. Tolbutamide Response

Blood sugars. In these experiments 26 subjects were maintained on standard diets and then were pretreated with Decadron. In some cases the same subject was retested with Prednisone in order to determine whether the two steroids were comparably diabetogenic. The effect of steroids on the tolbutamide response may be seen in FIGURE 6. Two types of response were noted. One curve, which was shown by 18 subjects, was termed normal since it most closely resembled the shape of the original or nonsteroid tolbutamide response. The second type of response, termed abnormal, was shown by 8 subjects; it faithfully reproduced the curve seen in diabetics. It is noteworthy that the response to steroids is manifested in all subjects by a delay in the time of maximal blood sugar depression. In the normal curve the delay is from 30 min. to 45 to 60 min., while in the abnormal curve, even at 90 min. the blood sugar is still falling. In addition to the time delay and change in shape of the curve, absolute changes in blood sugar level were noted following administration of steroids. At the 30 min. point, in the normal curve, approximately one third of the subjects showed a fall in blood sugar level of less than 23 per cent, namely, a diabetic level. The abnormal curve was characterized by all subjects showing a fall at 30 min. which was less than 23 per cent.

FIGURE 7 shows the absolute blood sugar levels and the ranges observed in all the subjects studied. Several points may be noted. First, there is a stepwise increase in the initial blood sugar level as seen by comparing the three curves. Second, the actual fall in blood sugar in the normal steroid curve is as great, or greater than that noted in the initial tolbutamide curve. This is not true in the case of the abnormal curve. Examination of FIGURE 8 shows the distribution of blood sugar values and indicates that a diabetic curve is to be expected following steroids if the initial blood sugar is above 115.0 mg. per cent or if the 30- and 45-min. samples are above 96.0 and 86.0 mg. per cent, respectively. In view of the overlap of blood sugar levels in the two curves, the combined observations of the shape of the curve and the level of blood sugar are required to call a response abnormal. In 3 subjects tested with Prednisone and Decadron, both steroids produced comparable changes. It is important to note that all subjects who gave an abnormal test with steroids showed a normal test with tolbutamide alone.

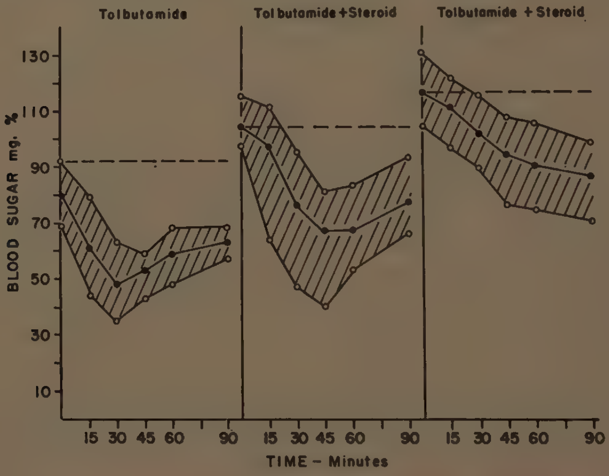

FIGURE 7. Mean and range of blood sugar values following tolbutamide with and without steroid pretreatment.

Nonesterified fatty acids (NEFA) of blood. FIGURE 9 shows the nonesterified fatty acid changes, subsequently referred to as NEFA, observed during the course of the blood sugar responses previously noted. Aside from the higher initial level of NEFA in both groups treated with steroids, there is a remarkable similarity in the shape and magnitude of response in all three curves. The fact that the abnormal sugar curve was associated with a normal NEFA response seemed unusual in view of the demonstrated relationship to NEFA of blood sugar responses.

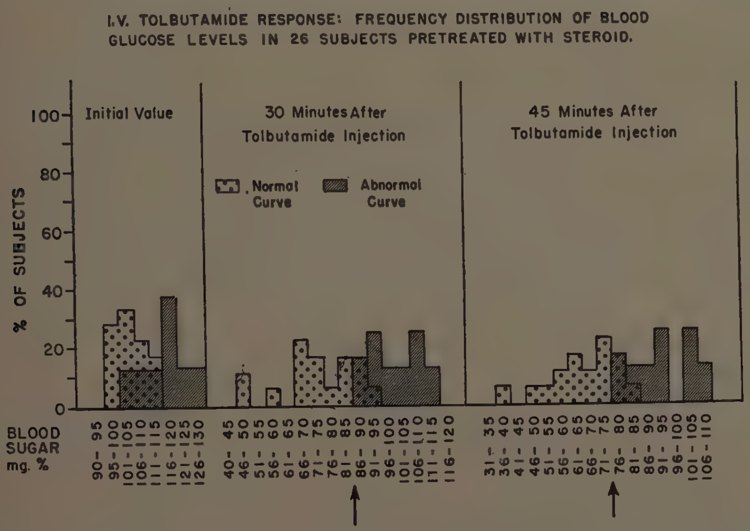

FIGURE 8. The position of the arrow indicates the level of blood sugar above which all curves were abnormal.

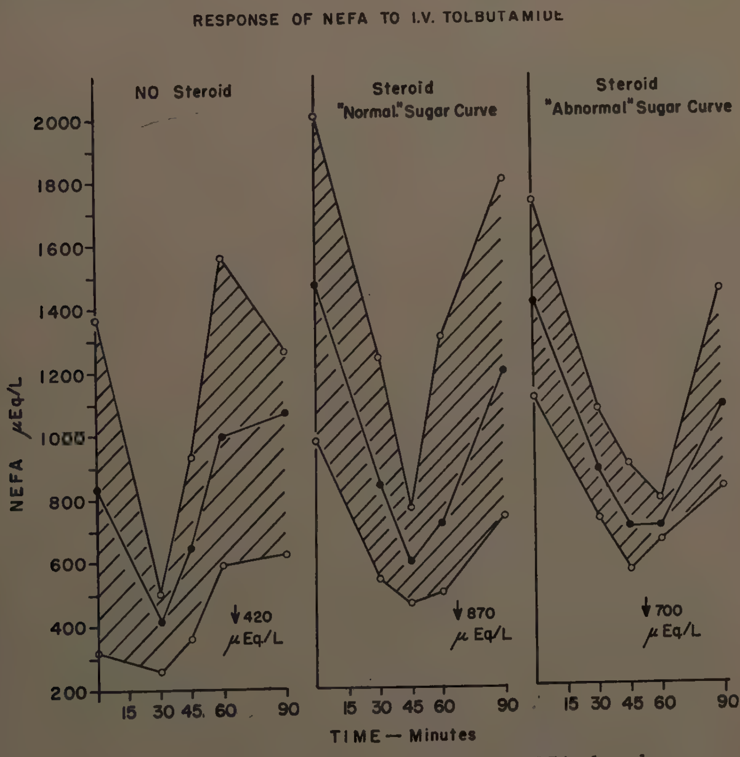

FIGURE 9. Mean values and range of NEFA plotted.

Relationship to family history of diabetes. The abnormal blood sugar curve in response to steroids was observed in 8 subjects. Of this group 6 gave a positive family history of diabetes.

DISCUSSION

The immediate response to intravenous tolbutamide appears to depend upon the release of insulin from the pancreas. The data obtained from studies using dogs also suggest that a simultaneous decrease in hyperglycemic factor may occur and contribute to this effect. It is of interest to note that the response appears to be short-lived and declining by the 30 min. point. However, since no observations were made after 30 min., it is not possible to state whether insulin secretion is sustained or reactivated therafter.

On the basis of these data obtained from animal studies, one can attempt to interpret the observations made using human subjects. It seems likely that following intravenous tolbutamide, the initial fall in blood sugar represents insulinlike action and this is noted in all normal subjects. The simultaneous fall in NEFA noted is similar to the one produced by insulin and suggests a peripheral action of the released insulin in adipose tissue. Following this, the normal individual responds with counter-regulatory mechanisms and both the blood sugar and NEFA levels rise. This secondary rise probably represents an epinephrine effect. Holzbauer and Vogt[9] have shown in the dog given insulin that epinephrine in peripheral blood is released almost as soon as the blood sugar starts to fall and continues to be released throughout the fall, gradually decreasing in quantity as the blood sugar rises. It is interesting that their data show that there is a less pronounced, but definite rise in epinephrine with as small a blood sugar fall as 15 mg. per cent. An additional point of interest relates to the demonstration that epinephrine causes a rise[10] in NEFA. These observations appear to explain the characteristics of the normal blood sugar and NEFA response to tolbutamide.

It is interesting that 24-hour starvation or excessive carbohydrate intake resulted in no changes in tolbutamide response. On the other hand, restriction of carbohydrate, known to induce a decrease in pancreatic insulin[11] among other metabolic effects, resulted in an alteration of the curve in one subject who later under stress of steroids gave a diabetic response. This suggests that restriction of carbohydrate is diabetogenic in the susceptible individual.

When steroid pretreatment was given, the tolbutamide response was significantly modified. Since the major effect of these steroids (Decadron, Prednisone) is to increase gluconeogenesis and raise the blood sugar level, it is not surprising that elevated blood sugars were observed in all patients. It seems likely that this rise in blood sugar occurring over an 8½-hour period prior to the tolbutamide injection is accompanied by an increased release of the stored insulin of the pancreas. Injection of tolbutamide at this time presumably tests the pancreatic reserve and the individual with diminished reserve shows a significantly smaller fall in blood sugar. This was apparent in the 2 types of curves noted and one might use the term prediabetic for the curve suggesting decreased insulin stores.

However, does the decreased reserve of insulin adequately explain the different shape of the curve, namely the continual decline of blood sugar in the prediabetic and diabetic? Since the NEFA curves were similar in the normal and prediabetic groups, showing a secondary rise, it seems probable that epinephrine was liberated in both groups. If the level of epinephrine required to increase NEFA is less than that required to restore the blood sugar to normal levels, it could be assumed reasonably that more epinephrine was released in the normal than in the prediabetic or diabetic. This could explain the failure to see a secondary rise in the abnormal sugar curves.

On the other hand, progression from the initial tolbutamide curve to the steroid-normal curve and, ultimately, to the steroid-prediabetic curve (FIGURE 6), is manifested by a delay in the time of maximal blood sugar fall. This delay could represent the conversion of preinsulin to insulin, when the stores of pancreatic insulin have been depleted and the pancreas is restimulated. If this were so, one might be measuring this slower process in the diabetic or in the prediabetic who manifests this change following steroids.

These challenging questions are unanswered and revolve about pancreatic metabolism and the mechanism by which tolbutamide effects a release of pancreatic insulin.

The data obtained relative to prediabetics is based on numbers too small, and follow-ups too short to be conclusive. However, the family history of diabetes appears to increase the probability of an abnormal tolbutamide response following steroids.

SUMMARY

Studies have been presented that were designed to explain the nature of the normal, prediabetic, and diabetic blood sugar response to intravenous tolbutamide.

Acknowledgments

We thank C. J. O'Donovan for supplying the sample of sodium tolbutamide and N. Capeci for the Prednisone and Decadron and Mary B. Koch and Eudoxia Hatch for their technical assistance.

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RELATIVE EFFECTIVENESS OF NEWER ORAL AGENTS IN THE REGULATION OF DIABETIC PATIENTS IMPERFECTLY CONTROLLED BY TOLBUTAMIDE STUDIED WITHIN THE FRAMEWORK OF A TENTATIVE SUBCLASSIFICATION OF THE DISEASE

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Specific correction of the underlying physiological derangement or deficit is the ultimate goal in therapy of disease. Such specificity in treatment requires the precise subclassification of the disease state along clinical and physiological lines. In diabetes the need for such subclassification has been largely neglected.

In the present study, we have attempted to evaluate, within the framework of a tentative clinicophysiological subclassification of diabetes, the relative effectiveness in this disease of the presently available oral blood glucose-lowering agents, both singly and in combination.

Subclassification of Diabetes Mellitus

It is generally agreed that diabetes can be divided into at least 2 major groups (TABLE 1): The first group, designated as Type 1, is perhaps best described clinically by the term ketoacidosis-resistant,* and defined as that segment of the diabetic population in which deprivation of all exogenous insulin will not, by itself, precipitate ketoacidosis under normal circumstances. This clinical type is characterized physiologically by the presence of insulin in the beta cells[2] and by insulinlike activity of endogenous origin[3,4] in the plasma.

Type 2 diabetes, more commonly referred to as juvenile, growth-onset, unstable, or labile diabetes, is perhaps more aptly described as ketoacidosis prone, to indicate that insulin withdrawal will, by itself induce ketoacidosis, coma, and death. This group is, of course, characterized by a virtual absence of insulin in the beta cells[2] and of endogenous insulin activity[3,4] in the plasma and might, therefore, be described as "abinsulinogenic."

No further consideration will be made in this report of this latter group nor of those transitional cases that are difficult to fit into either of these two categories.

Subclassification of Type 1 Diabetes

In order to provide a basis for clinical comparison, Type 1 diabetics have been arbitrarily subclassified into 3 subgroups according to the responsiveness of their mean fasting blood glucose level to tolbutamide therapy (TABLE 1). The speculative matter of the possible physiological implications of such a subclassification will be discussed below.

Patients in whom mean fasting normoglycemia is maintained with tolbutamide are designated as the tolbutamide-responsive subgroup; those in whom a blood-glucose response to maximal doses of tolbutamide takes place but falls short of fasting normoglycemia are designated as the tolbutamide-hyporesponsive subgroup. In this category blood sugar control ranges from near-normal to entirely unacceptable levels. Finally, those Type 1 diabetics in whom no significant blood sugar-lowering effect attributable to tolbutamide therapy can be discerned, are designated tolbutamide-unresponsive.

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* This term, recently proposed by Wilder,[1] seems preferable to the more commonly used terms, stable, adult, or maturity-onset diabetes.

Selection of Patients

The tolbutamide-responsive subgroup, as defined above, is ideally controlled according to usual clinical criteria. Mean fasting normoglycemia has been achieved without any penalty in the form of serious hypoglycemia or drug toxicity. Furthermore, as will be pointed out later, it is possible to defend this therapy on physiological grounds. Since the need for additional methods of blood sugar control seems least in this tolbutamide-responsive subgroup, the patients selected for this study were drawn primarily from the ranks of the tolbutamide-hyporesponsive and tolbutamide-unresponsive subgroups (93 per cent).

TABLE 1
CLASSIFICATION OF DIABETES

Type 1 Ketoacidosis-resistant			Type 2 Keto-acidosis-prone
Tolbutamide responsive (FBS normal on tolbutamide administration)	Tolbutamide hyporesponsive (partial response ranging from near normal FBS to unacceptably high levels)	Tolbutamide unresponsive (no detectable FBS response to tolbutamide)	

The patients were members of the Parkland Memorial Hospital Metabolic Clinic or the Veterans Hospital Diabetes Study Clinic and consequently were observed at weekly intervals as outpatients.

Methods

Weekly fasting blood glucose levels were determined in Parkland Hospital patients by a modification of the ferricyanide method of Hoffman,[5] using the Technicon Corporation Autoanalyzer.*

Therapy with chlorpropamide, metahexamide, and Phenformin (DBI) was begun at minimal dosage levels and the dose was raised progressively each week until a response in fasting blood glucose level (FBS), the appearance of drug toxicity, or an unsatisfactory response to the drug was observed. Persistence of FBS levels at or above the tolbutamide baseline values despite 4 to 6 weeks of a drug in maximal doses was considered to warrant change to another agent. The order of agents used following discontinuation of tolbutamide therapy was not randomized and was, in general, as follows: metahexamide, chlorpropamide, DBI, and tolbutamide plus DBI.

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* Kindly performed by the laboratory of Morton F. Mason.

The mean FBS level of each patient while on tolbutamide therapy was compared with the mean FBS level while on the various newer therapeutic regimens. Percentage of change in mean FBS level following conversion from tolbutamide to a newer oral drug was then calculated.

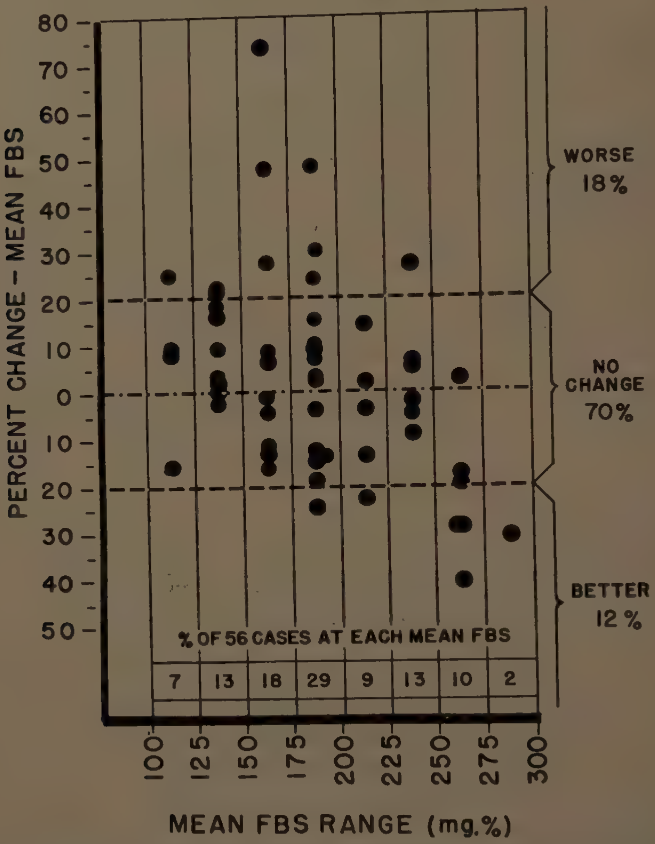

FIGURE 1. Change in mean FBS levels: metahexamide versus tolbutamide. Per cent change in mean FBS level in 56 diabetic patients imperfectly regulated with tolbutamide consequent to conversion from the latter agent to metahexamide. The patients are segregated according to the range of their mean FBS level on prior tolbutamide therapy indicated along the horizontal axis; the per cent change associated with metahexamide therapy is recorded on the vertical axis. A change in the mean FBS of 20 per cent or more is considered significant.

Results

Metahexamide. Fifty-six patients were changed from tolbutamide to metahexamide therapy. Metahexamide therapy was begun at dosage levels of 100 to 150 mg./day, administered in divided doses before breakfast and dinner. The dose was raised each week by 50 mg. until either a decline in FBS to normal or near-normal levels was noted, drug toxicity appeared, or 4 to 6 weeks of therapy at a dosage level of 300 to 400 mg./day had proved to be ineffective.

In FIGURE 1, on the horizontal axis, the range of mean FBS level during prior tolbutamide therapy is indicated for each of these patients; the per cent change from these levels during metahexamide therapy is shown on the vertical axis. A change in mean FBS level of less than 20 per cent was regarded as of doubtful clinical significance. Thirty-nine patients (70 per cent of this series) were therefore considered unchanged after conversion to metahexamide therapy; 7 patients (12 per cent) showed a reduction in mean FBS level from that of tolbutamide therapy, and 10 patients (18 per cent) exhibited a rise.

TABLE 2

MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO METAHEXAMIDE WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide
1*	145 (110 to 180) 296 (220 to 335)	188 (175 to 213)
2	272 (238 to 282)	194 (101 to 280)
3	179 (160 to 190)	138 (123 to 152)
4	293 (263 to 203)	197 (194 to 264)
5	266 (284 to 222)	160 (153 to 189)
6	201 (150 to 253)	156 (129 to 186)
7	186 (152 to 249)	140 (114 to 166)

* This patient had had 2 separate courses of tolbutamide therapy, the first of which was satisfactory, the second unsatisfactory. Conversion to metahexamide was preceded by an interval of insulin therapy.

The blood sugar range of the 7 patients who showed a significant reduction in mean FBS level while on metahexamide is examined more closely in TABLE 2. In all but the first patient, in whom interpretation is difficult because of a long hiatus between tolbutamide and metahexamide courses, the improvement over tolbutamide control appears to be genuine and clinically significant, although in no case can the resulting blood sugar control be considered ideal.

Of uncertain significance is the observation that improvement in blood sugar control following conversion to metahexamide therapy was most prevalent among patients in whom the mean FBS level while receiving tolbutamide had been highest. Conversely, deterioration in control appeared to be most common among patients whose mean FBS level had been normal while on tolbutamide. This circumstance imparts to FIGURE 1 the suggestion of a somewhat linear scatter, a pattern observed with the other agents as well (FIGURES 2, 3, and 4).

Chlorpropamide. Twenty-four patients were converted from tolbutamide to chlorpropamide. The drug was administered in a single daily dose beginning at 500 mg./day. The dose was increased by 125 to 250 mg./wk. until a decline in FBS to normal or near-normal levels, the appearance of drug toxicity, or 4 to 6 weeks of unsatisfactory FBS response at dosage levels of up to 1.0 gm./day had been noted. FIGURE 2 indicates the per cent change in mean FBS from tolbutamide baseline levels noted during therapy with chlorpropamide. Improvement was noted in 3 patients (12.5 per cent), deterioration in 5 patients (20 per cent), and in 16 patients (67.5 per cent) no significant change was observed. The range of fasting blood glucose levels of the 3 cases in whom improvement was noted is shown in TABLE 3. The improvement in these 3 cases, 2 of whom had been inadequately controlled by metahexamide as well as by tolbutamide, was striking, and in each case the FBS level was maintained within a clinically acceptable range.

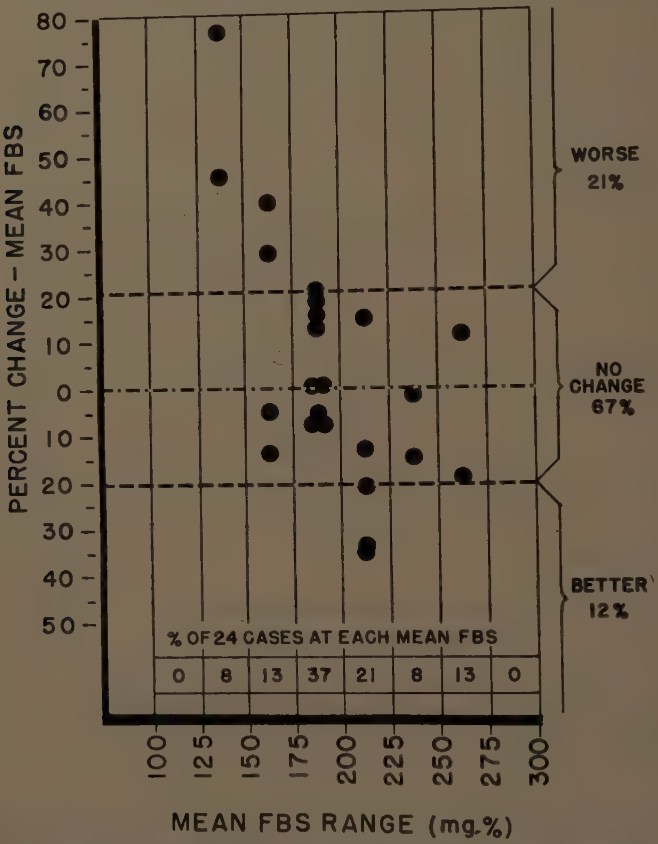

FIGURE 2. Change in mean FBS levels: chlorpropamide versus tolbutamide. The per cent change in mean FBS level in 24 diabetic patients imperfectly regulated with tolbutamide following conversion from the latter drug to chlorpropamide. The range of mean FBS level on prior tolbutamide therapy is indicated on the horizontal axis; the per cent change during chlorpropamide therapy is recorded on the vertical axis.

Phenformin. Twenty-seven patients were converted from tolbutamide to

Phenformin* (DBI). The drug was administered initially in doses of 75 to 100 mg./day in 4 doses, taken during each meal and at bedtime. The dose was increased by 25 mg. at each weekly visit until optimal control of FBS was attained or until a "bad taste" or anorexia appeared. Failure to reduce the

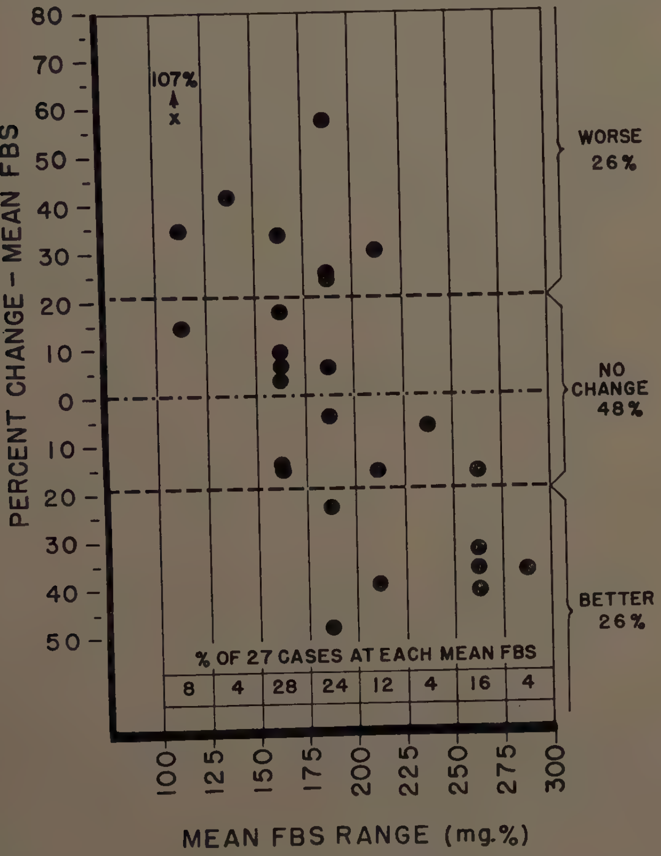

FIGURE 3. Change in mean FBS levels: Phenformin (DBI) versus tolbutamide. The per cent change in mean FBS level in 24 diabetic patients imperfectly controlled with tolbutamide following conversion to DBI. The range of mean FBS while on tolbutamide appears on the horizontal axis; the per cent change during DBI therapy is recorded on the vertical axis.

FBS level after 3 weeks of therapy at maximal subtoxic levels was considered evidence of drug failure.

Seven patients (26 per cent) showed significant improvement in mean FBS control after conversion from tolbutamide to DBI, 8 patients (30 per cent) appeared to deteriorate in this respect, while 12 subjects (44 per cent) were considered to be unchanged (FIGURE 3). The magnitude of improvement exceeded 30 per cent in 6 of the 7 improved cases.

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* Product of United States Vitamin and Pharmaceutical Corporation, New York, N. Y.

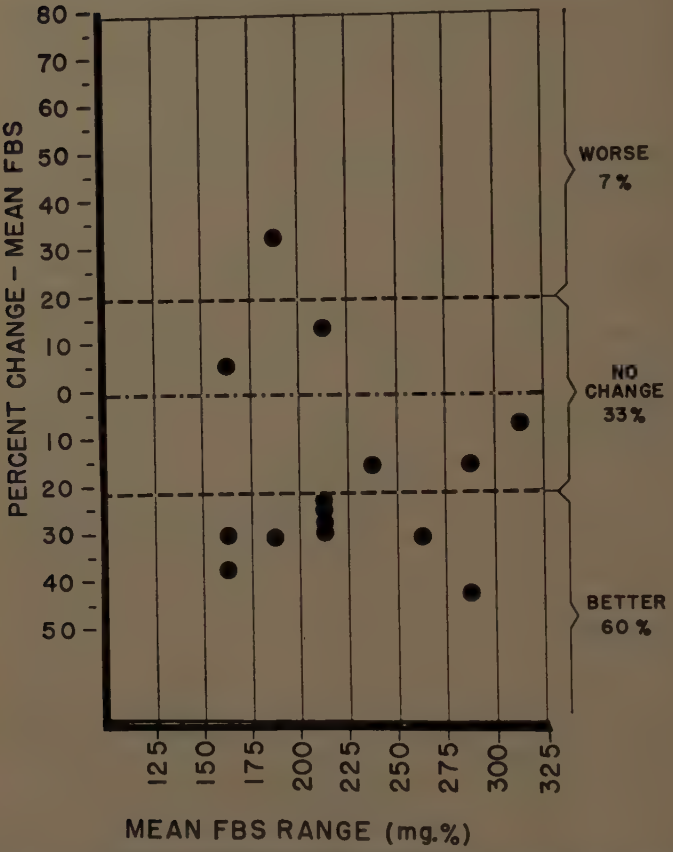

Figure 4. Change in mean FBS levels: combined DBI and sulfonylurea versus sulfonylurea alone. The per cent change in mean FBS level in 15 patients imperfectly controlled with tolbutamide following conversion to tolbutamide-DBI therapy. The range of mean FBS while on tolbutamide appears on the horizontal axis; the per cent change during combination therapy is shown on the vertical axis.

Table 3

Mean and Range of FBS Levels in Patients in Whom Conversion from Tolbutamide to Chlorpropamide was Associated with Decline in Mean FBS of 20 Per Cent or More

Patient	Tolbutamide	Metahexamide	Chlorpropamide
1	207 (193 to 248)	214 (135 to 296)	163 (129 to 175)
2	202 (201 to 206)		132 (100 to 172)
3	218 (207 to 289)	250 (195 to 286)	144 (96 to 200)

TABLE 4 shows the individual ranges of blood glucose levels in the cases in which improvement was observed. The narrow range of FBS levels, the acceptable values of control, and the unsatisfactory results with previous therapy are particularly notable. In this numerically small series it would appear that, of the 3 agents studied, Phenformin is most likely to prove effective in lowering FBS levels of diabetics imperfectly controlled with tolbutamide.

Combination sulfonylurea-biguanide therapy. The use of DBI in conjunction with tolbutamide was studied in 15 patients, few of whom had been ideally controlled by any of the previous oral agents used singly. The order in which the drugs were administered varied. In some patients tolbutamide was added to the regimen of a DBI-unresponsive patient in whom an earlier trial of tolbutamide as well as the other sulfonylureas had failed to bring about acceptable blood sugar control. In other cases Phenformin was added directly to an unsuccessful tolbutamide regimen. In all, tolbutamide was considered the basic therapy (dose 1.5 to 3.0 gm.) and DBI the supplementary agent to be given in whatever subtoxic amounts might be required for optimal FBS control. The daily dose ranged from 75 to 250 mg. in this study.

TABLE 4

MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO PHENFORMIN (DBI) WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide	Phenformin
1	255 (201 to 300)	263 (201 to 291)	285 (251 to 300)	173 (144 to 192)
2	203 (125 to 250)	177 (131 to 201)	178 (80 to 268)	152 (124 to 178)
3	272 (261 to 282)	194 (101 to 280)	—	119 (101 to 157)
4	196 (135 to 280)	256 (150 to 350)	195 (136 to 265)	116 (102 to 127)
5	189 (120 to 273)	236 (90 to 470)	—	146* (127 to 188)
6	262 (110 to 335)	188 (175 to 213)	—	169 (154 to 183)
7	294	—	—	189 (189 to 200)

* Since the preparation of this table this patient has become unresponsive to Phenformin.

Short-term observations of from 3 to 12 weeks' duration revealed substantial improvement in mean FBS level over that of prior tolbutamide therapy in 60 per cent of these patients, no change in 33 per cent, and deterioration in only 7 per cent (FIGURE 4). The means and ranges of FBS levels in the improved cases are shown in TABLE 5.

Toxicity. The toxicity data are summarized in TABLE 6 and require little amplification.*

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* The data on toxicity are based on observations in a larger number of patients than are included in the evaluations of comparative effectiveness. These additional patients, although receiving various of the newer agents, could not be included in the comparative studies for want of an adequate baseline period on tolbutamide therapy.

The side effects of DBI, which are confined to the gastrointestinal tract, are not included in TABLE 6 since these symptoms will invariably appear when the limits of individual tolerance are exceeded. These vary widely from patient to patient (75 to 400 mg. in our experience). In this respect the drug is similar to digitalis.

It should be stressed that in TABLE 6 the term hypoglycemic symptoms refers to symptoms compatible with hypoglycemia that were relieved by food and does not imply laboratory documentation. Although the danger of hypoglycemia during therapy with tolbutamide alone is slight, and with Phenformin *alone* probably absent, its incidence when these 2 agents are used in combination is as yet uncertain.

TABLE 5

MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO TOLBUTAMIDE-PHENFORMIN COMBINATION THERAPY WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide	Phenformin	Tolbutamide-Phenformin
12*	294	—	—	189 (184 to 200)	125 (97 to 156)
13*	185 (136 to 210)	193 (163 to 245)	170 (135 to 180)	220 (144 to 250)	133
11*	293 (263 to 308)	197 (144 to 270)	—	249 (242 to 256)	130
4	255 (201 to 300)	263 (255 to 291)	284 (251 to 300)	172 (144 to 192)	119 (80 to 139)
5	172 (95 to 250)	—	—	—	126 (116 to 135)
6	234 (146 to 421)	300 (164 to 396)	—	259 (190 to 348)	149 (140 to 158)
7	188 (137 to 238)	181 (124 to 225)	189 (124 to 220)	181 (103 to 240)	134 (129 to 140)
8	215 (177 to 300)	—	—	—	156 (140 to 168)
9	206 (199 to 215)	171 (111 to 202)	250 (224 to 275)	—	155 (115 to 186)
3	202 (181 to 222)	—	132 (100 to 172)	—	150 (117 to 177)
1	270 (269 to 285)	219 (151 to 300)	220 (195 to 217)	277 (216 to 280)	167 (140 to 188)
2	191 (148 to 261)	—	—	—	157 (144 to 178)
10	272 (267 to 278)	—	—	—	136 (114 to 158)

* Not included in FIGURE 4 because of inadequate baseline observation periods.

During both metahexamide and chlorpropamide therapy, serial determinations of alkaline phosphatase levels revealed in 24 and 25 per cent, respectively, of patients followed, a level of 7 Bodansky units (B.U.) or above on at least 1 occasion. The peak value occurring in each such patient is recorded in FIGURE 5. In many of these cases the elevation in alkaline phosphatase level was transient, returning to normal despite continued therapy, often to recur. In some, however, a progressive rise, at times to alarming levels, was observed. As a check on the technical accuracy of the clinical laboratory a blood specimen was

TABLE 6

INCIDENCE OF SIDE EFFECTS DURING THERAPY WITH THE LONG-ACTING SULFONYLUREAS

	Metahexamide (82 patients)	Chlorpropamide (37 patients)
Hypoglycemic symptoms	4 (5)%	2 (5%)
Nausea	7 (9%)	2 (5%)
Vomiting	2 (2.5%)	0
Abdominal pain	2 (2.5%)	1 (2.5%)
Diarrhea	0	2 (5%)
Weakness	0	2 (5%)
Rash	1 (1.3%)	2 (5%)
Drug fever	1 ?(1.3%)	0
Jaundice	1 (1.3%)	0
Elevation in alkaline phosphatase > 7 B.U.	(24%)*	(25%)*

* See FIGURE 5.

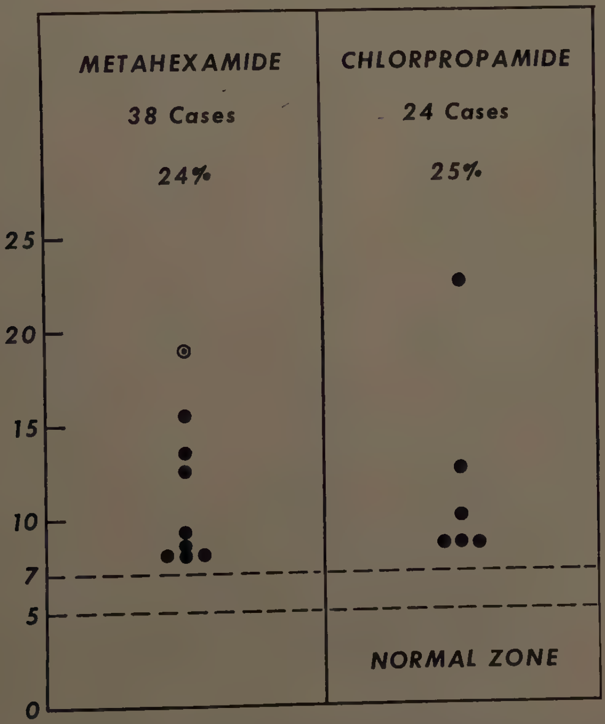


FIGURE 5. The distribution of alkaline phosphatase levels over 7 Bodansky units in 38 metahexamide-treated patients in whom serial determinations were made. The many patients not included did not have any determinations of alkaline phosphatase. The values charted represent the highest level obtained in each patient. The circled dot represents the jaundiced patient described in the text.

In 40 patients receiving tolbutamide and/or DBI the highest level of alkaline phosphatase obtained was 4.9 Bodansky units (the vertical axis is in Bodansky units).

obtained from each of 40 patients receiving Phenformin and tolbutamide. The highest alkaline phosphatase level in this group was 4.8 B.U.

In 1 patient, a 52-year-old Negro female receiving metahexamide in doses of 100 to 350 mg. over a 10-week period, evidence of clinical liver disease was

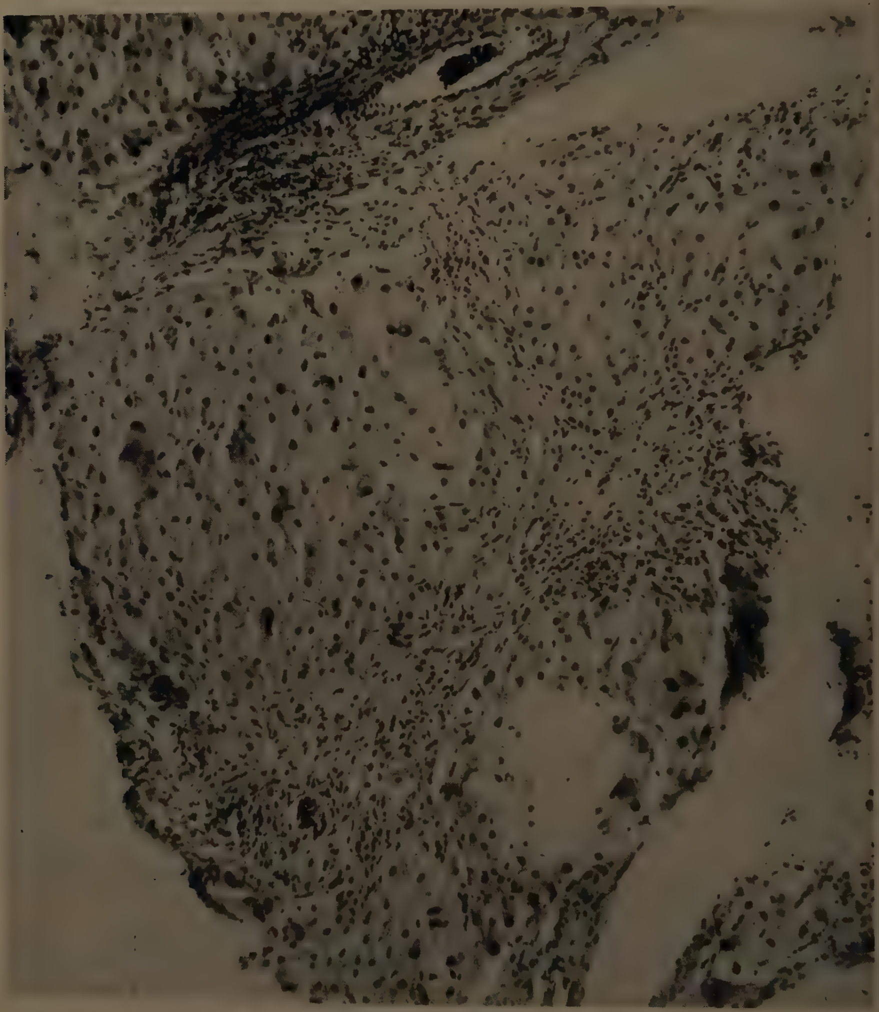


FIGURE 6. Sections of the needle biopsy of the liver reveal portions of several nodular areas of liver tissue. The nodular foci are rounded, about 0.5 mm. in size, and surrounded by a zone of collagenous fibrous tissue. The hepatic cells are moderately swollen and pale, showing considerable hepatocellular regeneration. The nodular regenerating areas of liver cells fail to show normal architecture. Central veins are not identified, and the cords are in disorderly array. No marked dilation of bile canaliculi is observed, and there is no retention of bile pigment within the canaliculi. However, several moderately visible canaliculi are visible, some of which contain eosinophilic, inspissated-appearing, finely granulated material. The fibrous stroma surrounding the nodular foci is rather loose and contains numerous capillaries. There is moderate infiltration of this area by lymphocytes and rare polymorphonuclears. Courtesy of Charles T. Ashworth.

noted. This patient was hospitalized because of anorexia, malaise, abdominal pain, and jaundice. Serial liver biopsies, kindly studied by Charles T. Ashworth, were considered to show postnecrotic cirrhosis of probable recent onset (FIGURE 6), attributable either to viral hepatitis or to a drug reaction. This patient's recovery has been uneventful.

Discussion

Interpretation of results. Clinical data must always be interpreted in the light of the host of variables that may influence studies of this kind. In addition to a certain idiopathic variability in the severity of diabetes mellitus, the possibility of spontaneous alteration in diet, of unrecognized infection, and of psychic factors, is most difficult to evaluate in outpatients and must be considered in the interpretation of these results. Furthermore, the anorexigenic effect of certain of the oral agents, DBI in particular, may in some patients contribute to blood sugar-lowering action via reduction in food intake. Finally, the number and duration of observations must weigh heavily in the formulation of conclusions.

Substantial improvement in control of mean FBS level occurred in only 12 per cent of patients imperfectly regulated by tolbutamide following conversion to metahexamide or chlorpropamide therapy, an unimpressive salvage rate. The possibility of acute or chronic cholangiolar and hepatic injury by these agents seems to preclude, at least for the present, their use for routine control of blood sugar, save in the exceptional case in which no other regimen, oral or parenteral, is effective.

DBI alone appeared to have a higher salvage rate of (26 per cent—7 patients) among tolbutamide-hyporesponsive patients. The magnitude of the improvement in FBS in these 7 patients was considerably greater than that noted in the case of metahexamide and chlorpropamide, ranging from 30 to 50 per cent in 6 of the 7 improved cases. Whether this improvement in FBS level was brought about via physiologically acceptable mechanisms is, at the moment, unknown. There is presently, however, no information to indicate that the blood sugar-lowering action of DBI is harmful.

The early results with tolbutamide plus DBI are impressive. Nine of 15 patients (60 per cent), all but 1 of whom had been inadequately controlled by 1 or more of the oral agents alone, have been acceptably controlled for 3 to 12 weeks on combination therapy. Because of the small size of this series and the short period of observation any final conclusion in respect to this group must be deferred. The initial results, however, are most encouraging (FIGURE 4).

Physiological implications of tolbutamide-responsiveness. The seeming incongruity of a near-normal pancreatic insulin content and plasma insulin activity in a patient with persistent hyperglycemia can be explained in more than one way. Lacking incontrovertible evidence of interference with insulin effectiveness or of its inactivation or neutralization, the following concept seems most reasonable. The functional lesion in tolbutamide-responsive diabetics may well consist of a hyporesponsiveness in terms of insulin release per unit of time to a given level of betacytotropism. Thus, in order for such a beta cell to maintain a normal insulin output, an elevated blood sugar level is required (FIGURE 7)*. Tolbutamide, in some way, may diminish this reluctance of the beta cell to release its insulin, thereby permitting a normal insulin output at a normal rather than at an elevated blood sugar level. The drug may thus provide a highly physiological approach to therapy in this type of diabetes. The use of tolbutamide in the tolbutamide-responsive subgroup can be defended on

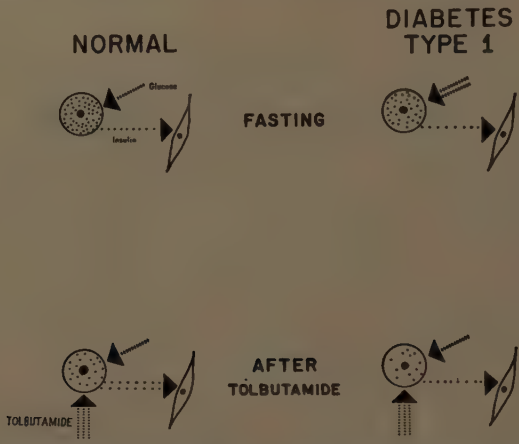
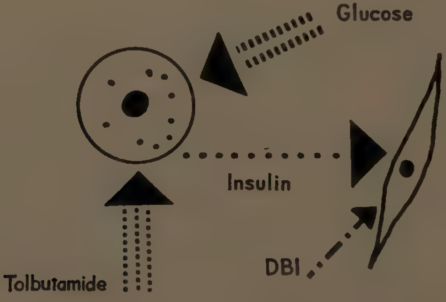


FIGURE 7. The apparent incongruity of a normal insulin-output in the presence of an elevated blood sugar is schematically illustrated in the upper panel. It has been hypothesized that in certain diabetics a normal insulin-output can be maintained only when the blood glucose level is elevated. After tolbutamide a normal insulin-output may prevail, with a normal blood sugar level. This hypothesis would explain certain of the experimental and clinical observations in the tolbutamide-responsive Type 1 diabetic and would endow tolbutamide therapy in this group of patients with physiological justification.



Tolbutamide plus DBI

FIGURE 8. Type 1 diabetes (tolbutamide-hyporesponsive). In the tolbutamide-hyporesponsive Type 1 diabetic, beta-cell insulin content and output is presumably somewhat diminished. Tolbutamide therapy alone increases it, but not to normal values; hence the peripheral action of DBI is used to provide the additional removal of glucose needed to normalize the blood glucose level.

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* Since preparation of this manuscript, H. S. Seltzer and W. Smith have reported evidence to support this hypothesis.[6]

both clinical and, tentatively, on physiological grounds and may well be the treatment of choice for this category of diabetes.

The difference between the tolbutamide-hyporesponsive subgroup and the tolbutamide-responsive group may be purely quantitative, or additional defects may be present. In any case a more potent sulfonylurea or betacytotropin of established safety and superior effectiveness would seem appropriate. Until such an agent is available, the use of tolbutamide as the basic therapy with Phenformin supplementation, seems to offer the best clinical promise and to be physiologically acceptable (FIGURE 8).

The defect in the tolbutamide-unresponsive subgroup may be the result of a severely reduced insulin output by beta cells working at maximal capacity and incapable of further increase in function, as has been suggested by others.[7] In this group exogenous insulin must, of course, continue to be the mainstay of therapy, although supplementation with one or more of the oral agents may be helpful.

Summary and Conclusions

Only 12 per cent of patients imperfectly controlled with tolbutamide undergo substantial improvement in mean FBS levels when converted to metahexamide or to chlorpropamide. Increase in the level of alkaline phosphatase was noted in 25 per cent of the patients receiving these agents. Postnecrotic cirrhosis developed in 1 metahexamide-treated patient. Until the risk of cholangiolar and hepatic damage by these agents has been finally clarified, their routine use would seem to be precluded.

DBI alone resulted in substantially improved control in 26 per cent of patients imperfectly regulated by tolbutamide. In highly preliminary results combined tolbutamide-DBI therapy resulted in improvement in control in 60 per cent of a small series.

An attempt has been made to develop a clinicophysiological subclassification of diabetes based on responsiveness to tolbutamide. This may facilitate clinical comparison of new agents and fill, at least temporarily, the nomenclatural vacuum that prevents the ultimate goal of the precise physiological differentiation of diabetes prerequisite for specificity in therapy.

Acknowledgment

We express our gratitude to Charles T. Ashworth, Chairman of the Department of Pathology, University of Texas Southwestern Medical School for the pathological examination reported herein, and to Morton F. Mason for the laboratory work on the Parkland Hospital Patients.

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COMBINED INSULIN-TOLBUTAMIDE THERAPY IN THE MANAGEMENT OF INSULIN-DEPENDENT DIABETES

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Early observations on the sulfonylurea compounds made by German clinicians indicated that these substances may be successfully used in the management of mild diabetes of the maturity-onset type, but are of no benefit in more severe forms of the disease and in patients who acquired it at an early age. Since the compounds were found to be ineffective in depancreatized and alloxan-diabetic animals it was concluded that for their antidiabetic activity they need an adequate islet reserve and that, therefore, their use is contraindicated in the growth-onset type of diabetes and in patients who require large doses of insulin. At the same time, however, it was also noted that about one third of patients with the maturity-onset diabetes who were considered to be good candidates for the use of sulfonylureas failed to respond to these compounds. The question of why these patients were refractory to sulfonylurea preparations remained unanswered.

From unpublished experimental work with 2254 R.P., the compound used by the French investigators in their original studies of hypoglycemic thiodiazols, we have gained the impression that the presence of an intact pancreas does not insure a hypoglycemic response to the employed thiodiazol and that some extrapancreatic factors may be involved in the mechanism by which this thiodiazol exerts its influence on the blood sugar content. With these thoughts in mind we assumed that failure of diabetic patients to respond to the newer sulfonylureas may be related to extrapancreatic factors and have decided to use these compounds in conjunction with insulin in patients whose diabetes cannot be controlled without the employment of this hormone. Because these studies were begun shortly after the compounds became available in the United States,[1,2] we are able to present observations that extend over a period of more than three years and include a large series of patients in whom the use of these compounds is generally thought to be contraindicated.

Clinical Material and Results

Table 1 presents the clinical data on our group of 131 patients with insulin-dependent diabetes. To test the feasibility of combined insulin-Orinase therapy in such patients we have selected patients with labile diabetes who, because of the notorious vagaries of this type of diabetes, cannot be well controlled even with the most ingenious and meticulous schemes of insulin administration. Figure 1 illustrates our tribulations during early phases of such therapy, and in particular the failure of Orinase to prevent rapid deterioration of metabolic control in these patients when their insulin dosage is reduced by as little as 2 or 4 U. daily.

Since diabetes is a chronic disease, long-term effects of an antidiabetic medication seemed to us more important than the immediate effects noted in acute experiments. Furthermore, we thought it more profitable to study an antidiabetic medication not only as a substitute for insulin, but on its own merits. For these reasons we have continued the use of Orinase in our labile diabetics in spite of the discouraging outcome of short-term observations. In subsequent patients the administration of the drug was initiated without a change in insulin dosage, but even with this regimen it took from 2 to 8 weeks before an improvement from the combined therapy became clearly noticeable. A favorable response was obtained in 35 patients who, thus far, have been continued on the combined therapy for periods of 12 to 40 months. All 35 experienced stabilization of their diabetic control together with a feeling of general stability and well-being. In 21 of them there was also a substantial saving in insulin dosage that is reflected in gradual reduction by 27 to 50 per cent of the pretreatment dose. The remaining 11 patients with labile diabetes were not improved by adjunctive administration of Orinase. In 9 of them the drug was discontinued after 3 to 6 months of therapy. Two patients given Orinase developed paradoxical hyperglycemia and increased glycosuria which necessitated an increase in their insulin dosage. Since such disruption of metabolic control occurred in three widely separated trial periods of 14 to 18 days, the use of the drug was abandoned after the short-term experiments.

TABLE 1

CLINICAL DATA ON 131 INSULIN-DEPENDENT DIABETICS GIVEN COMBINED INSULIN-ORINASE THERAPY (C.I.-O.T.)

No. of cases	Clinical type	Diabetes		Insulin prior to Orinase (units per day)	Response to C.I.-O.T.	
		Age at onset (years)	Duration (years)		Good	Poor
46	labile	6 to 40	4 mo. to 34 yr.	28 to 132	35* (76%)	11‡ (24%)
27	stable mild	39 to 75	8 mo. to 24 yr.	10 to 40	22** (81.5%)	5 (18.5%)
58	stable severe	11½ to 56	5 to 29	52 to 156	51† (88%)	7§ (12%)

* In 21 patients the insulin requirement decreased by 27 to 50 per cent.
** In all patients insulin requirement decreased by 25 to 70 per cent.
† In all patients insulin requirement decreased by 10 to 65 per cent.
‡ Two patients developed paradoxical hyperglycemia and increased glycosuria.
§ Patients with onset of diabetes between 7 and 11 years of age.

In the group of stable diabetics given combined insulin-Orinase therapy, there were 27 with mild diabetes who required from 10 to 40 U. of insulin daily. Ten of these patients were initially well controlled on Orinase alone, but after 2 to 4 months broke out of control and were compelled to add small amounts of insulin to the Orinase therapy. Six other patients felt surprisingly well for periods of up to 10 months, even though their glycosuria continued in large amounts (up to 40 gm. daily). The remaining 11 patients of this group exhibited deterioration of diabetic control even with minor reductions in insulin dosage, and in consequence were not taken off insulin entirely at any time. Five of these mild diabetics derived no benefit from the combined therapy and, in consequence, the administration of Orinase in these patients was discontinued.

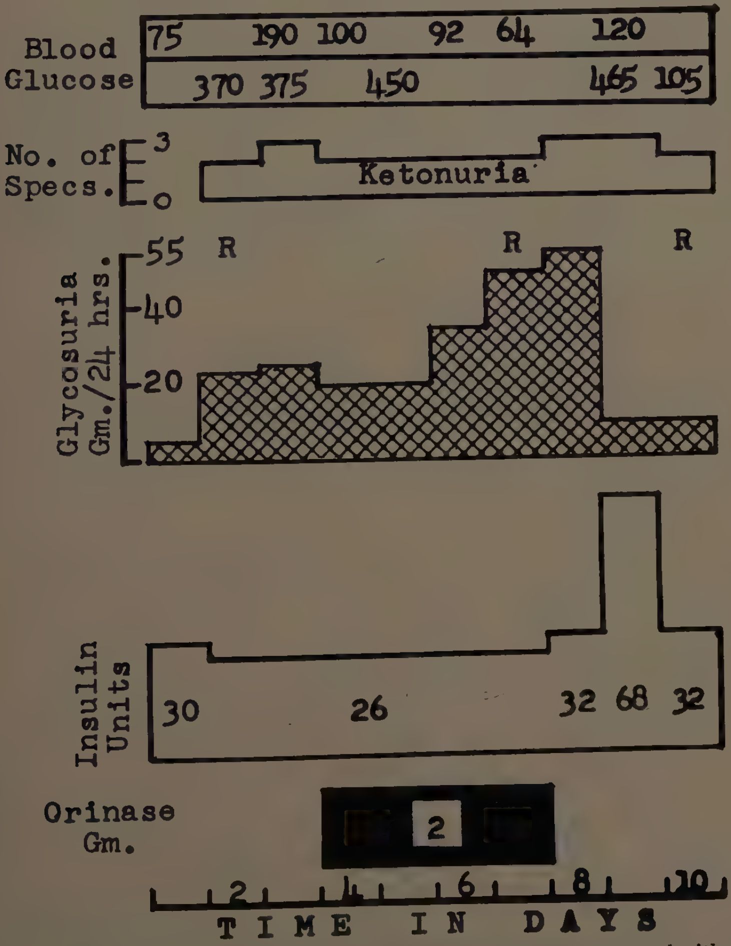

FIGURE 1. Effect of partial withdrawal of insulin in labile diabetes treated with Orinase. Blood glucose values (mg. true glucose per 100 ml. venous blood) were determined in the fasting state (*lower line*) or 2 hours after a meal (*upper line*). Four urine specimens were collected daily, three between 7:00 A.M. and 7:00 P.M. and one between 7:00 P.M. and 7:00 A.M. Ketonuria is graded by the number of urine specimens that showed a positive reaction for ketone bodies. "R" designates moderately severe insulin reactions in the afternoon of the indicated days. The diagram illustrates rapid deterioration of control following a small reduction in insulin dosage and failure of Orinase to influence this acute loss of control. The patient, a 44-year-old white woman with onset of diabetes at the age of 10, needed up to 132 U. of insulin at the age of 25. She received between 34 and 44 U. daily in the 3 months preceding the above trial of combined therapy. Orinase therapy was restarted, without changing the insulin dosage, 10 days after discharge from hospital and has been continued thus far for 16 months. Within 4 weeks on the second trial of combined insulin-Orinase management she obtained satisfactory control and stabilization of her diabetes. At present she receives 26 U. of insulin and 1.5 gm. Orinase daily. Reproduced by permission from *Metabolism, Clinical and Experimental.*

Among the 58 patients with severe diabetes, 7 with onset of the disease between 7 and 11 years of age showed minimal or no response to Orinase. Even after 8 to 12 weeks on Orinase therapy, they still reacted with disruption of metabolism when their usual insulin dosage was decreased but slightly. By contrast, patients with a high insulin requirement who acquired the disease after the age of 30 were greatly benefited by the use of Orinase. The saving in insulin in these patients ranged from 10 to 65 per cent of the pretreatment dose, and all demonstrated a smooth control of their diabetes and a feeling of general stability and well-being.

Discussion

In our series of 131 insulin-dependent diabetics beneficial effects from long-term combined insulin-Orinase therapy were obtained in 35 patients (76 per cent) with labile and 74 patients (86 per cent) with stable diabetes. The benefits consisted of: (1) a sense of general stability and an enhanced feeling of well-being; (2) stabilization of diabetes in labile patients and ease and greater uniformity in diabetic control in both groups; (3) a decrease in insulin requirement of up to 50 per cent of the pretreatment dose in the labile and of up to 70 per cent in the stable group; and (4) a decrease in frequency and severity of hypoglycemic reactions in all patients including those whose insulin dosage remained unchanged.

It is important to point out that greater uniformity in diabetic control and the general feeling of stability and well-being were not related to changes in insulin dosage. Such benefits were seen in patients who achieved a marked reduction in their insulin requirement as well as in those with little or no decrease in their pretreatment insulin dosage. These facts indicate that the beneficial effects from adjunctive Orinase therapy are not confined to the hypoglycemic action of this substance and that the use of Orinase in combination with insulin provides benefits that cannot be attained with insulin alone.

By what mechanism Orinase achieves the stabilizing and mood-raising effects is at present unknown. It is, of course, tempting to postulate that the compound stimulates the production or release of endogenous insulin and in this way makes it possible to reduce or even to eliminate the administration of exogenous insulin and in so doing to obtain a smoother metabolic control. However we must keep in mind the fact that the feeling of stability and well-being associated with the use of Orinase is also seen in patients who have never been treated with insulin, and therefore it cannot be ascribed to cessation of insulin therapy in these patients. As mentioned earlier, such general benefits are also seen in patients on combined insulin-Orinase therapy whose insulin requirement was not lowered by taking this sulfonylurea, that is, in patients who show no evidence of stimulation of islets by the compound. We interpret these observations as indicative of extrapancreatic effects of sulfonylureas, particularly in relation to carbohydrate transformations in extrapancreatic body tissues.

Summary and Conclusions

Long-term effects of Orinase as an adjunct to insulin therapy were studied in a group of 131 insulin-dependent diabetics for periods of 12 to 40 months.

Beneficial results were obtained in 76 per cent of 46 patients with labile and 86 per cent of 85 patients with stable diabetes. Outstanding among such benefits were a feeling of general stability and well-being, and ease and greater uniformity in metabolic control. A saving in insulin dosage ranging from 10 to 70 per cent of pretreatment dose was also achieved in a large number of patients, but the described general beneficial effects were not related to changes in insulin dosage.

Observations herein reported and the fact that one third of patients regarded as good candidates for therapy with sulfonylureas are refractory to these compounds are taken to indicate (1) that responsiveness to sulfonylureas does not seem to depend exclusively on the pancreatic islets and (2) that extrapancreatic factors are operative in bringing about the clinical improvement in patients treated by the combination of insulin and Orinase.

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PHYSIOLOGICAL BASIS OF THE EFFECTIVENESS OF COMBINED INSULIN-TOLBUTAMIDE THERAPY IN STABLE DIABETES*

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Considerable physiological evidence has accumulated suggesting that the pancreatic beta cells are the main target for the hypoglycemic action of the sulfonylurea drugs. Loubatières originally demonstrated that these compounds lower the blood sugar in the normal and in the partially pancreatectomized dog but not in the completely depancreatized nor in the severely alloxan-diabetic animal.[1] Increased insulin output from the beta cells under the influence of these drugs is suggested also by experiments in which systemically ineffective dosages perfused directly through the pancreatic artery caused significant peripheral hypoglycemia.[2] Furthermore, in cross-circulation experiments hypoglycemia was noted in recipient animals after perfusion of the pancreatic arteries of donor dogs with the sulfa drugs.[3] Moreover, an increased insulin content of the pancreatic vein in the dog has been reported following acute administration of the sulfonylureas.[4] In more recent experiments it was shown electrophoretically that there is an increase of insulin in the pancreatic vein blood of calves after the administration[5] of D 860.

There is also, however, evidence that there may be extrapancreatic actions of these drugs which affect the blood sugar level. Thus chronic administration of tolbutamide is reported by some to reduce the degree of diabetes of completely depancreatized dogs maintained on small dosages of insulin.[6] Others could not demonstrate a significant action of these compounds on the rate of blood glucose removal, while the rate of conversion of lactose to glucose was diminished.[7] Furthermore, the rate of disappearance of intravenously infused pentose was shown to be increased threefold after insulin, but was not affected by the sulfonylureas.[8] Several workers have found a decrease of pyruvate and lactate after carbutamide, compared with an increase after insulin.[9,10] Still other investigators utilizing C^{14}-tagged glucose observed that after insulin administration in the rat there is a rapid fall in specific activity in the blood while, after tolbutamide, the fall in specific activity is no greater than in control animals.[11] Furthermore, it has been shown that there may be a marked increase in blood sugar in rabbits following the rapid intravenous injection of tolbutamide, usually terminating in death of the animal.[12] Extrapancreatic mechanisms that have been suggested to account for these findings include, particularly, enhancement of insulin action by inhibition of insulinase[13] and inhibition of hepatic glycogenolytic systems,[14,15] as well as some action on the central nervous system.[12]

Morphologically it has been shown, unequivocally, that when administered over prolonged periods the sulfonylureas produce degranulation of the pancreatic beta cells of rats, rabbits, and dogs (FIGURE 1).[16-19] The functional significance of beta-cell degranulation might be twofold. Theoretically the

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* The work reported in this paper was supported in part by a grant from The Upjohn Company, Kalamazoo, Mich.

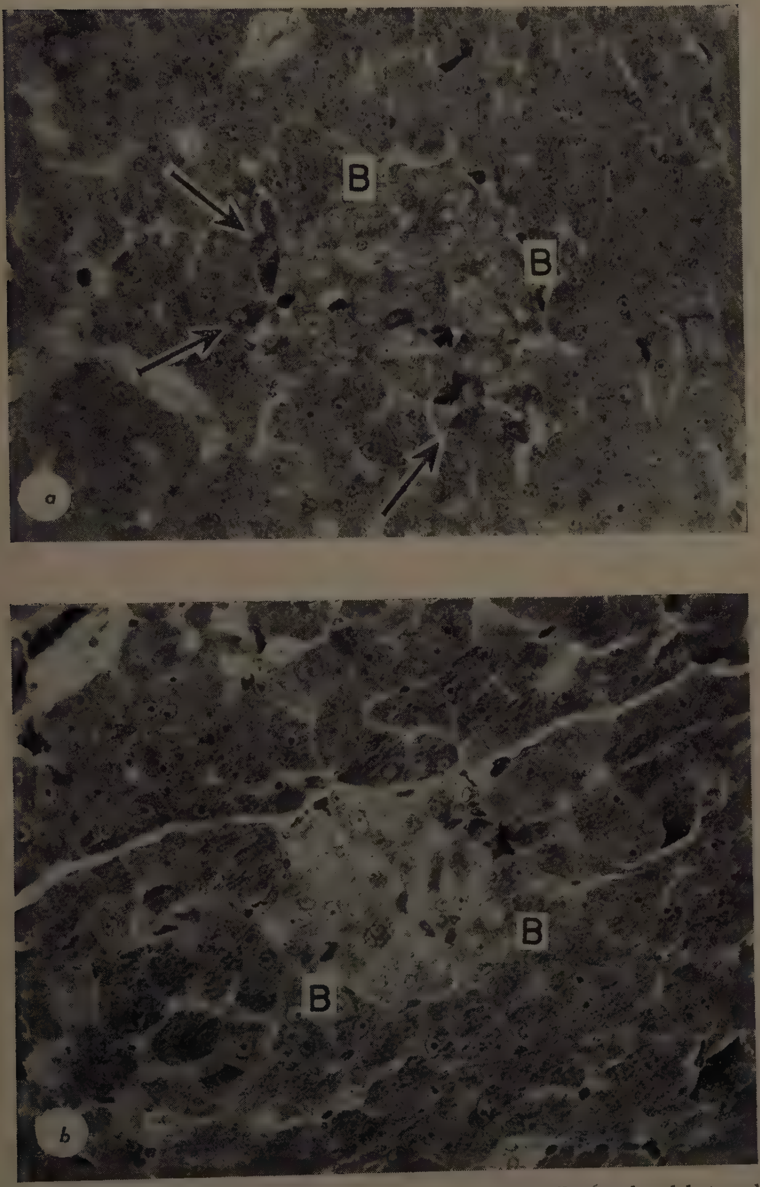

Figure 1. (*a*) Islet of pancreas of normal dog showing well granulated beta cells (B). Alpha cells (*arrows*) are dark. Aldehyde-fuchsin stain. (*b*) Islet of pancreas of dog who received 1.0 gm. of tolbutamide orally, twice daily for 35 days. The beta cells (B) are almost completely degranulated. The alpha cells are dark. Aldehyde-fuchsin stain. ×380.

granules, which represent a reserve of stored insulin, may be diminished either by decreased production or increased output of insulin from the beta cells.

Experimentally it has been shown that in the rat fasting, a high fat diet, or administration of exogenous insulin caused beta-cell degranulation and reduced pancreatic insulin content.[20-23] In these instances it is assumed that the beta-cell degranulation is indicative of suppression of insulin formation. On the other hand, hyperglycemia due to glucose administration in the rat also causes beta-cell degranulation and diminished pancreatic insulin content.[24-26] Under the latter circumstances it is believed that although insulin synthesis is increased, the rate of liberation of insulin from the pancreas is so high that the amount of stored insulin, as represented by the granules, is reduced.

The degranulation of beta cells after sulfonylureas has been interpreted by some as indicative of suppression of insulinogenesis similar to that observed during chronic hypoglycemia induced by insulin administration.[16] The main argument advanced in favor of this theory was that beta-cell degranulation is not observed after very short-term administration of these drugs. However, in both the cortisone-treated rabbit[27] and the growth hormone-treated dog,[28] degranulation of beta cells which in both instances is due to increased insulin output also is not observed within the first 48 hours. An additional argument advanced to support the view that tolbutamide degranulation is due to depressed insulinogenesis is that it causes no histochemically demonstrable depletion of pancreatic islet zinc content.[29] However, this conclusion is not warranted since little is known as to the actual relationship between insulin, zinc, beta-cell granules, and alpha cells. Furthermore, this finding could be interpreted as disproof either of the suppression or the stimulation hypothesis.

Relation Between Tolbutamide-Induced Beta-Cell Degranulation and Hypoglycemia

In order to determine whether tolbutamide-induced degranulation of pancreatic beta cells was a primary effect of the drug or whether it was secondary to the sulfonylurea-induced hypoglycemia, 2 types of experiments were conducted. In the first type, hypoglycemia equivalent to that produced with a standard dose of 0.5 gm./kg. of tolbutamide b.i.d. was produced in rabbits by administration of 3 U. of NPH insulin daily (FIGURE 2). After 7 days the animals were sacrificed and the extent of beta-cell granulation of the pancreas was compared. It was found that although tolbutamide produced varying degrees and frequently complete degranulation of the pancreas, insulin produced no changes in the beta cells in the rabbit. In the second type of experiment, rabbits were infused through the marginal ear vein for 7 hours on 3 successive days with approximately 300 cc. of 5 per cent glucose in saline, or a similar perfusate to which 625 mg./kg. of tolbutamide or metahexamide had been added. The added sulfonylurea caused no decline of blood sugar values below the normal fasting level (FIGURE 3). The animals which received glucose and the drug simultaneously showed, in most instances, complete loss of beta-cell granules (FIGURE 4). The rabbits that received glucose alone showed no beta-cell changes (FIGURE 5).

The results of these two experiments indicate that the hypoglycemia is not

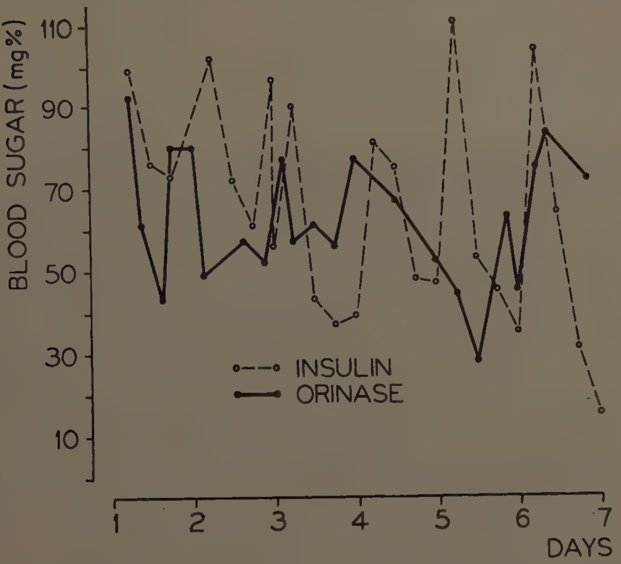

FIGURE 2. Representative blood sugar curves of 2 rabbits, 1 treated with 0.5 mg./kg. of tolbutamide intravenously twice daily, the other with 3 U. of NPH insulin subcutaneously once daily. Reproduced by permission from *Diabetes.*

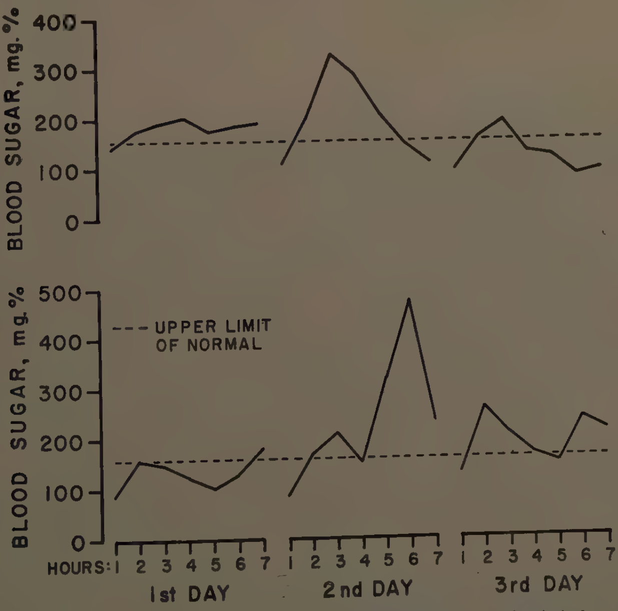

FIGURE 3. Representative blood sugar curves of 2 rabbits, 1 (*top*) infused through the marginal ear vein for 7 hours on 3 successive days with 300 cc. of 5 per cent glucose in saline alone; the other (*bottom*), with a similar amount of glucose in saline to which 625 mg./kg. (2 gm.) of metahexamide had been added.

the cause of the beta-cell degranulation and, furthermore, that the drugs produce degranulation even though hypoglycemia is prevented by simultaneous glucose administration. These findings are interpreted to mean that the sulfonylureas have a primary action on beta cells that increases the insulin out-

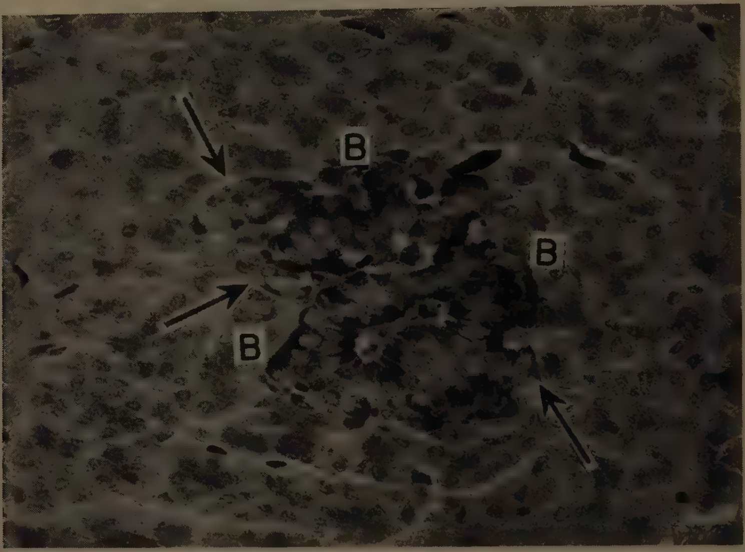

FIGURE 4. Islet of pancreas of rabbit infused with glucose alone showing normal beta-cell granulation (B). Alpha cells (*arrows*) are at the periphery. Aldehyde-fuchsin-trichrome stain. × 380.

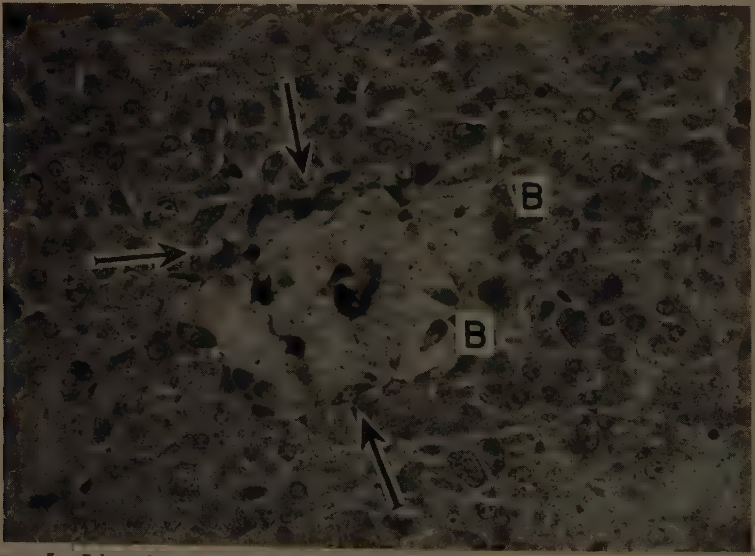

FIGURE 5. Islet of pancreas of rabbit infused with glucose and metahexamide showing degranulation of beta cells (B). Alpha cells (*arrows*) are dark. Aldehyde-fuchsin-trichrome stain. ×380.

put and, thereby, causes degranulation. This increased insulin output then would account for the drug-induced hypoglycemia.

Relation Between Hypoglycemic Effectiveness of Tolbutamide and Pancreatic Insulinogenic Reserve

It was hypothesized that since the beta cell is the primary target of the sulfonylureas if these cells are functioning at maximum capacity, administration of the drug should cause no further increase in insulin output.[12] This viewpoint was supported by the fact that during chronic administration of tolbutamide an additional single test dose of the drug was found to be ineffective in further lowering the blood sugar level.[30] To test this hypothesis, studies were conducted on the effectiveness of the drug in experimental diabetes induced by reducing the pancreatic mass and by administration of cortisone or growth hormone. It has been shown that in certain phases of these hyperglycemic states the functional reserve of the pancreas is exhausted.[27,31,32] For this purpose rabbits were treated with large dosages of cortisone and 80 per cent partially pancreatectomized dogs received growth hormone or cortisone. It was found in these experiments that the blood sugar response to tolbutamide decreased significantly with increasing severity and duration of the diabetic state. It was also found, with one exception, that in rabbits or dogs in which beta cells were completely degranulated, administration of a single dose of the drug did not cause hypoglycemia (FIGURE 6). Furthermore, in metahypophyseal diabetic dogs, in which the pancreatic beta cells had been destroyed, daily administration of tolbutamide caused no improvement of the diabetic state.[12] These experiments support the view that the hypoglycemic action of the sulfonylureas is mediated by stimulation of beta cells to increased insulin output and that with loss of pancreatic functional reserve the drugs become ineffective.

Clinical Considerations

The relative effectiveness of sulfonylureas in various types of human diabetes supports the idea that the pancreas is the primary target of these drugs. Human diabetes mellitus is thought of as sometimes being due to lack of insulin and also frequently as being due to extrapancreatic factors that increase the demand for insulin or interfere with its action.[33-35] Juvenile diabetes, which is frequently of the insulin-deficiency type usually does not respond to the sulfonylureas, and often the pancreas shows loss of islet tissue (FIGURE 7). Patients with maturity-onset diabetes may have considerable insulinogenic reserve with adequate, or only moderately diminished islet tissue (FIGURES 8 and 9) and do respond to the drug.[36,37] Furthermore, it has been shown that many adult diabetics show good beta-cell granulation, as well as adequate extractable pancreatic insulin.[38] The fact that some diabetics may be controlled with drugs that stimulate the beta cells suggests that these individuals have a defect in the normal mechanism for controlling blood sugar by which hyperglycemia increases the insulin output.

With this hypothesis in mind, a study was conducted on the effect of tolbutamide alone or administered together with insulin in a group of patients with maturity-onset diabetes. Those selected usually had fasting blood sugars above 200 mg. per cent, despite the administration of at least 30 U. of insulin. It was found, in general, that they fell into two categories. In one group, after gradual reduction and eventual omission of insulin, the blood sugar remained within the normal range on 1 gm. of tolbutamide daily alone (FIGURE 10). In the other type of patient, normoglycemic levels were maintained on 1 gm. of tolbutamide together with approximately one half of the original dose of insulin (FIGURE 11). In the latter group the blood sugar rose to much higher values when insulin was omitted completely.

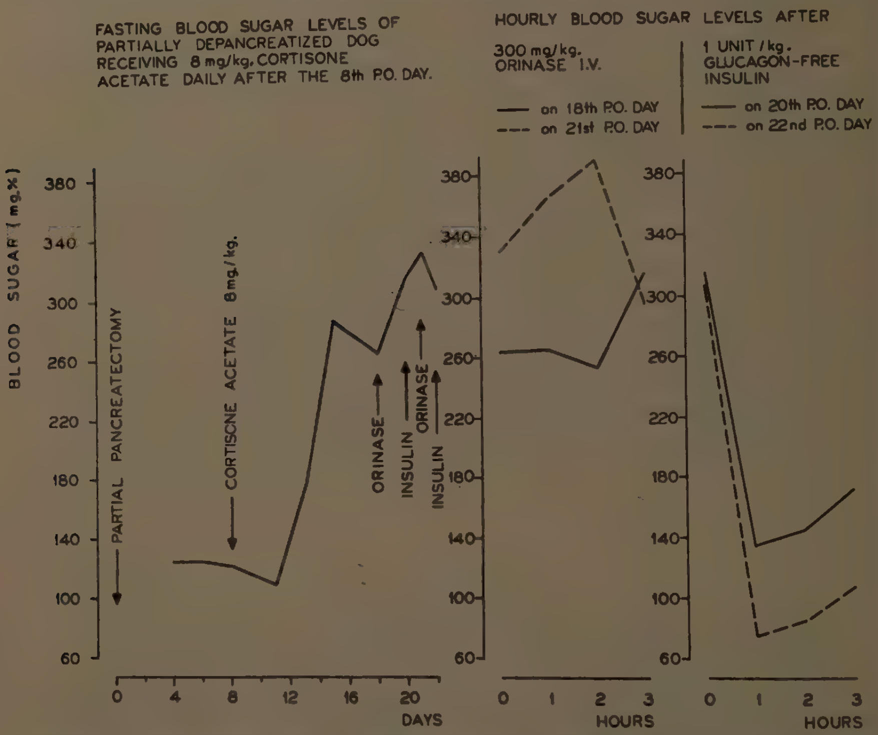

FIGURE 6. Blood sugar curve of a severely diabetic dog which received 8 mg./kg. of cortisone acetate intramuscularly daily starting on the eighth day after partial pancreatectomy (*left graph*). A single test dose of 300 mg. of tolbutamide on the eighteenth and twenty-first days caused no decline in the blood sugar (*middle graph*). The response to 1 mg./kg. of glucagon-free insulin is indicated in the right hand graph.

These studies demonstrate that tolbutamide administered together with insulin frequently has a synergistic effect in diabetic patients. This is in keeping with the reports of others who noticed beneficial effects of the supplemental use of these drugs in stable, as well as in labile diabetics with high insulin requirement.[39-46]

The clinical data as well as the experimental findings allow this synergism to be explained on the basis that exogenous insulin administered alone depresses its endogenous secretion, whereas when tolbutamide is administered together

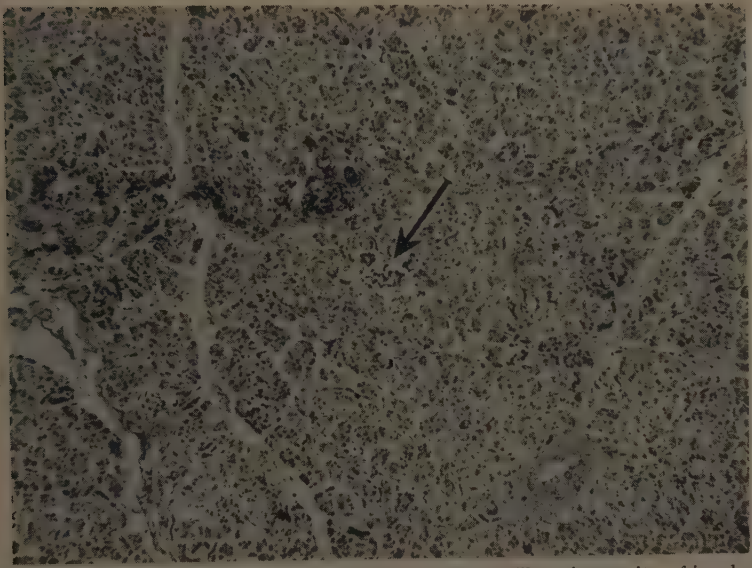

Figure 7. Pancreas of patient with juvenile diabetes. There is paucity of insular tissue (The pancreatic tissue was received through the courtesy of P. M. Le Compte). Aldehyde-fuchsin stain. ×95.

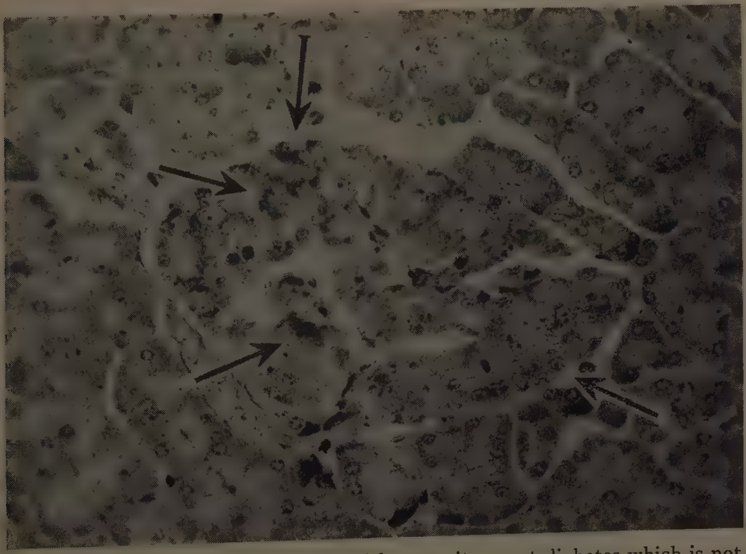

Figure 8. Islet of pancreas of patient with maturity-onset diabetes which is not readily distinguishable from the normal showing beta-cell granulation (*arrows*). Aldehyde-fuchsin stain. ×250.

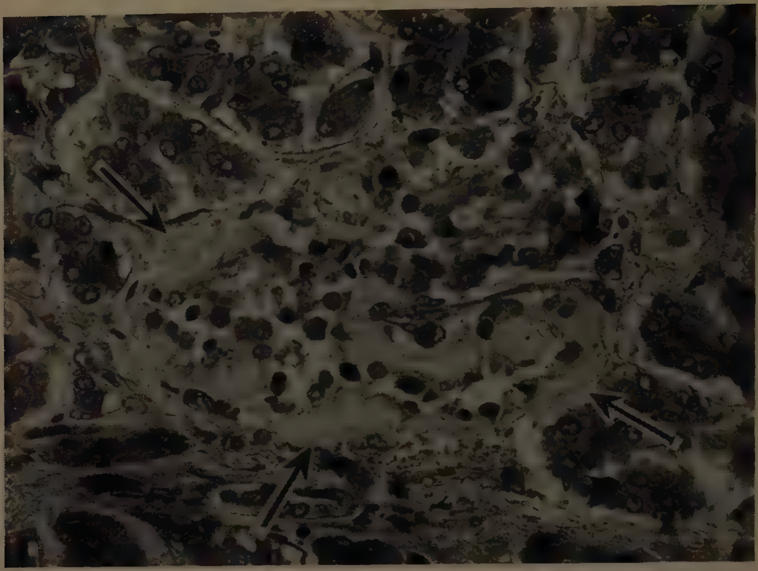

FIGURE 9. Pancreatic islet of patient with maturity-onset diabetes, showing partial hyalinization (*arrows*). Periodic-acid-Schiff-trichrome stain ×380.

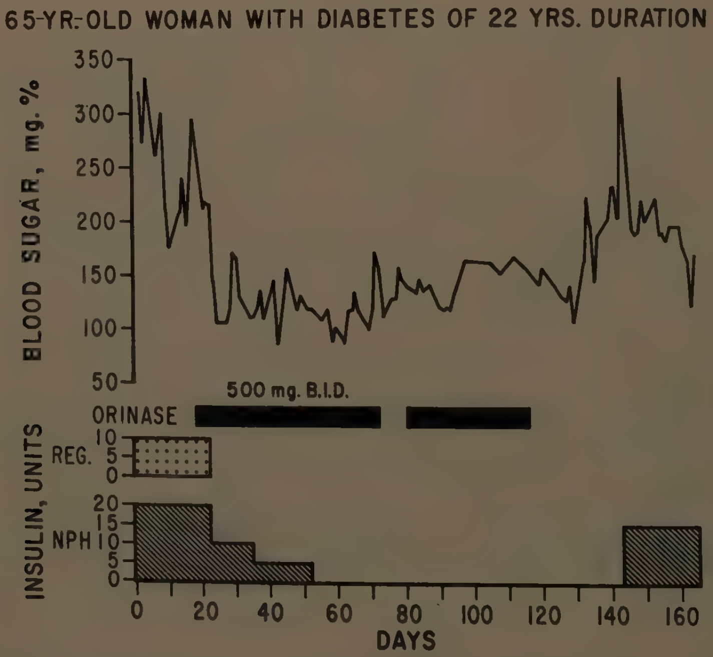

FIGURE 10. Blood sugar curve of diabetic showing better control with 1 gm. of tolbutamide daily alone than with a mixture of 20 U. of NPH and 10 units of regular insulin.

with insulin, endogenous production of the hormone is maintained. This apparently allows for smoother and better metabolic control of the diabetes than when insulin is given alone. The findings imply that the sulfonylureas apparently act as a more effective insulinogenic stimulus than does hyperglycemia and that in some instances diabetes may result from a loss of responsivity of the pancreas to increase in the blood sugar level. The gradual loss of effectiveness of the sulfonylureas reported in some diabetics[46] may be explained as due to maximal utilization in these individuals of the insulinogenic reserve which is, however, inadequate to maintain metabolic normality.

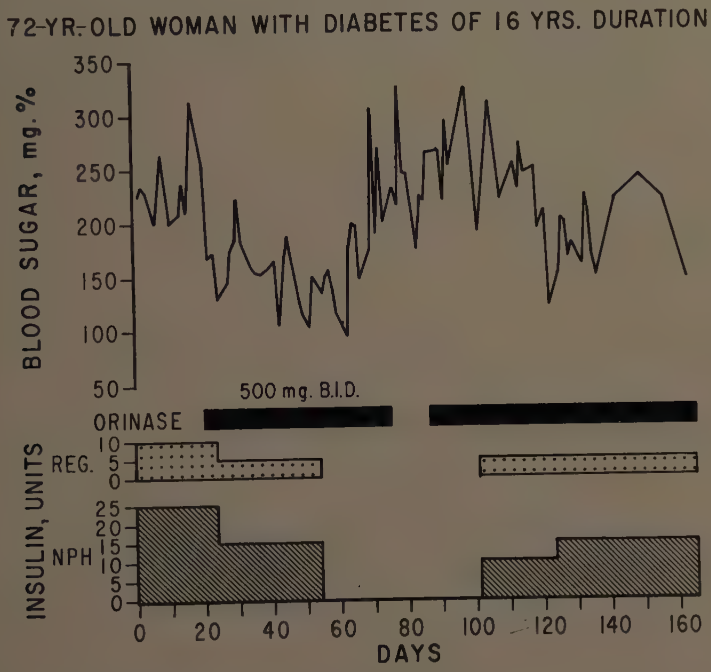

Figure 11. Blood sugar curve of diabetic showing better control with combined tolbutamide-insulin treatment than with either alone.

Summary

It has been demonstrated that the sulfonylureas cause degranulation of pancreatic beta cells. Furthermore, it has been shown that this pancreatic beta-cell degranulation is not due to the hypoglycemia which accompanies administration of the drug but is rather a primary effect of the drug on the beta cells. This degranulation is, therefore, considered to reflect increased insulin output from the beta cells under the influence of the drug. It was demonstrated furthermore that when the pancreas is functioning at maximal capacity and there is no insulinogenic reserve, as indicated by complete degranulation of the beta cells, the sulfonylureas do not cause hypoglycemia.

This finding is interpreted to mean that in order for the sulfonylureas to be effective, some pancreatic insulinogenic reserve must be present. With these ideas in mind a study was undertaken of the effectiveness of tolbutamide either alone or in combination with insulin in a group of selected diabetic patients with maturity-onset diabetes who were poorly controlled despite the fact that they received at least 30 U. of insulin daily. It was found that frequently good control could be obtained with a reduced amount of insulin together with a standard dose of 1 gm. of tolbutamide daily. On the other hand, occasional patients responded to the drug alone. The tolbutamide-insulin synergism in these patients is considered as signifying that tolbutamide stimulates endogenous pancreatic function despite the depressing effect of concomitant exogenous insulin. These findings suggest that most maturity-onset diabetics have varying amounts of residual pancreatic insulinogenic ability which is not utilized when they are treated with insulin alone. Furthermore, it is suggested that diabetic patients who respond to tolbutamide alone probably have adequate insulinogenic reserve but a defect in the normal mechanism for controlling the blood sugar by increasing the insulin output.

Acknowledgment

The microphotographs were prepared by Herbert A. Fischler.

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THE BIGUANIDES: THEIR ROLE IN THIS ERA OF THE PRECISE TOOL

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This "brave new world" of oral hypoglycemic agents is not of contemporary or even recent origin (FIGURE 1). In 1918 Watanabe[1] reported a correlation between the effect of guanidine on intermediary metabolism and decreased blood glucose levels. The intervening four decades have seen the rise and fall of many compounds. At present, there is interest in a freshet of oral agents whose common denominator is the ability to assist in lowering blood sugar levels. The sulfonylureas (carbutamide, tolbutamide, chlorpropamide, and metahexamide) have been and are being explored and delineated. The experience with biguanides is not as extensive, although their use in more than 3000 cases has been reported.[2] Pomeranze[3] first reported the use of phenethyl biguanide in human subjects. When it became apparent that, while the sulfonylurea compounds undeniably lowered blood sugar levels in many patients, their scope of action was clearly limited and offered no succor to the severe and brittle diabetic, our study series with biguanides started in December 1956.

The Biguanides

The biguanides used were phenethyl (DBI), *n*-amyl (DBB) and *n*-butyl (DBV) biguanide. These otherwise closely related analogues differ in degree of hypoglycemic activity and side effects. The dosage used was from 50 to 450 mg./day in divided doses, with an average effective dose of 150 mg./day. Certain patients also required a supplementary dose of insulin. The area between the effective dose and the level at which side effects occur proved to be a relatively narrow one in the more severe cases with unstable diabetes.

Material

In the past 28 months (December 1956 to April 1959) more than 350 patients received 1 or more doses of the biguanides. Of the first 300, 56 were deleted from the series (TABLE 1). Of the remaining 244 who were adequately studied, many were chosen because they were difficult to control as follows:

(1) One hundred eleven were growth-onset diabetics and 133 were adults whose onset of diabetes was later than age 16.

(2) Sixty were new diabetics (duration 1 year or less), while 184 were known to have diabetes 2 years or longer. Duration of diabetes was 1 to 37 years, while the average known duration was 9.4 years.

(3) Age of the 244 cases studied was 2 to 80 years (average 37.5 years; *see* TABLE 2).

(4) Thirty-two patients had never taken insulin previously. The other 212 averaged 32 units daily with a range of 4 to 136 units.

Methods

The routine of study and criteria used have been previously published,[4] and an extensive use was made of placebos to demonstrate the activity of diabetes.

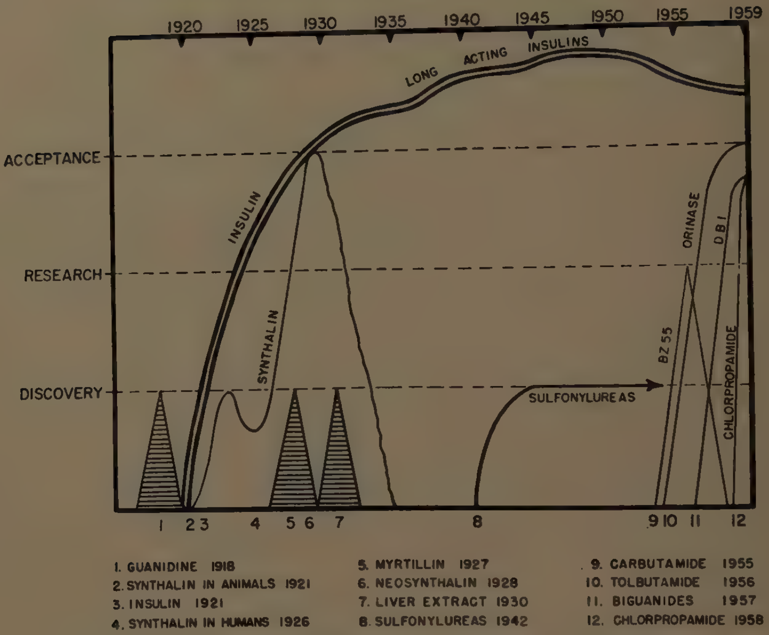

FIGURE 1. The rise and fall of the hypoglycemic agent dynasties.

TABLE 1
CLASS D: 56 CASES DELETED FROM STUDY

28	Eye study only (subhypoglycemic doses)
17	Inconclusive
8	Inactive diabetes (shown by placebo studies)
3	Uncooperative
56	TOTAL

TABLE 2
DISTRIBUTION OF AGES AT ONSET OF STUDY
(244 Cases (Groups A, B, and C))

Ages	Cases
0 to 9	26
10 to 19	41
20 to 29	28
30 to 39	27
40 to 49	28
50 to 59	45
60 to 69	35
70 to 80	12
80+	2
Total..................................	244

The criteria used were adequate but not perfect, since many factors may contribute to lowering of blood sugar levels, and diabetes is a disease of occasional remissions. The report of Balodimos[5] of 100 diabetic patients is a reminder of the long-known fact that hospitalization and strict diet can lower insulin dosage by more than 50 per cent. Reinforcement with placebo studies has made the criteria more acceptable.

Results

According to the classification in TABLE 3, the results are as shown in TABLE 4. Combining groups A and B shows that 88 per cent of the total of these selected difficult patients demonstrated blood sugar lowering, while adding groups B and C shows that 44 per cent of the 244 cases either did not tolerate the drug or failed to show success in the dose used.

TABLE 3

CLASSIFICATION OF CASES ACCORDING TO THE BLOOD SUGAR-LOWERING RESPONSE TO THE BIGUANIDES

Group 1—*Successful.*	A demonstrable blood sugar-lowering effect according to criteria established in a previous paper.[4]
Group 2—*Discontinued.*	This group had a demonstrable blood sugar-lowering effect but were unable to tolerate the drug. Discontinued from the series because of side effects.
Group 3—*Failure.*	No demonstrable blood sugar-lowering effect in the doses administered.
Group 4—*Not in study.*	This group includes uncooperative patients, inconclusive results, those under observation with minute doses as part of another study, and certain patients who could not demonstrate diabetic activity during the placebo phase of the study. These patients are not included in results unless they had side effects.

TABLE 4

RESULTS WITH 300 CASES RECEIVING BIGUANIDES

	No.	Per cent
Group A (*Successful*)	137	56
Group B (*Discontinued*)	79	32
Group C (*Failure*)	28	12
	244	100
Group D (*Deleted from study*)	56*	
Total	300	

* Includes 8 dropped from Group A because of no evidence of active diabetes.

Group D. This group consists of the 56 patients who were deleted from the study group of 300 (TABLE 1).

Group C. The 28 failures showed no pronounced characteristics. They were distributed equally among all age groups but were more prevalent in the longest-duration diabetics who required larger doses of medication (TABLE 5).

Group B. These were 79 patients who did not tolerate DBI in the doses used, although there was evidence of hypoglycemic action. The side effects were anorexia, 62; nausea, 56; vomiting, 29; diarrhea, 14; headache, 10. Some complained of a "metallic taste" but not severely enough to discontinue the drug. The side effects were all gastrointestinal and were readily reversed by lowering the dosage or discontinuing the drug. Except for temporary discomfort, no other consequence resulted from these side effects. The best prevention was and still is to give the pills with meals and to use perceptive clinical acumen, adjusting the dose prophylactically when needed. The problem of side effects may be the greatest deterrent to widespread use of these drugs if not judiciously prescribed.

TABLE 5
DURATION OF DIABETES AT ONSET OF STUDY*

137 cases—Class A successful ←		Compared with: 28 cases—class B failures →
1111111111 1111111111 1111111111 1111111111		1–1
2222222222 2222		
3333333		3
44444444		
5555555	Range: 1 to 37 years, average: 8 years.	
66666666		6
7777		777
8888		8
999		9
10		
11–11		11
12–12–12–12		12–12
13–13–13		13–13
14–14–14–14		14
15–15–15		15
16–16		16
17–17		18
19		19
20		20
21–21		21
22		
23–23		
25–25		25
26		
27–27		27
28–28–28		28
29–29	Range: 1 to 33 years	30
33	Average: 14.6 years	32
35		33
37		

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* Each number represents a separate case and indicates the actual duration in years of known diabetes.

Group A. According to previously cited criteria, 137 cases were classified as "successful." Comparing the ages of this successful group (A) with the total study group (A + B + C) in TABLE 6 shows that the attrition from the study in each age group is generally consistent throughout, but greatest in the age 10 to 19 bracket. The group also contains 27 patients who took no previous insulin. The 110 who previously took insulin averaged 44 units daily, with doses of 4 to 100 units.

After therapy was initiated, 62 patients used a daily average of 21 units of insulin daily with an average of 150 mg. of biguanide among total doses of 50 to 450 mg. daily. Seventy-five of these cases required no exogenous insulin supplement.

Long-term studies. One hundred and seven of the 137 successful cases (group A) continue with biguanides. Forty-nine are taking supplemental, although reduced, doses of insulin, while 58 are treated with the tablets alone. The duration of therapy is 3 to 28 months, with an average of 16 months. Seventy-five cases have continued DBI or DBB for 12 or more months, with a range of 12 to 28 months (average of this group, 22 months).

Toxicity studies. In the entire group at this time no toxicity has been manifested in liver function tests including Bromsulphalein retention, cephalin flocculation, thymol turbidity, alkaline phosphatase, or in the nonprotein nitrogen, white and red cell counts, hemoglobin, differential count, or complete urine analysis.

TABLE 6
COMPARISON: AGE AT ONSET OF STUDY
(Total Study Versus Successful Group)

Ages	Groups A, B, and C	Group A	Net loss
0 to 9	26	18	−8
10 to 19	41	16	−25
20 to 29	28	12	−16
30 to 39	27	13	−14
40 to 49	28	22	−6
50 to 59	45	31	−14
60 to 69	35	21	−14
70 to 79	12	4	−8
80+	2	0	−2
	244 cases	137 cases	107 (total B + C)

Experimental Considerations

The biguanides offer interesting and ubiquitous contrasts. They lower blood sugar levels in alloxanized and depancreatized animals as well as in diabetic humans. There is considerable doubt that the depancreatized animals could exist for any length of time with DBI alone, and Fajans[6] observed that DBI-induced hypoglycemia in diabetics but not in normal human subjects. Blood glucose levels are lowered and the glucose apparently utilized, but glycogen is not stored by the action of these compounds. Despite these random observations, the mechanism of action is not yet known.

Recently Steiner and Williams[7] summarized their belief that DBI action is based on increased anaerobic glycolysis and suggested that this effect is due to inhibition of certain oxidative enzymes, namely, cytochrome oxidase and succinic dehydrogenase.

Tranquada *et al.*[8] found no decrease in oxygen consumption in the liver during the hypoglycemic action of DBI, and no decrease in oxygen consumption or "availability" has been demonstrated in any tissue *in vivo.* Bolinger *et al.*[9] reported that DBI did not inhibit the deposition of glycogen by insulin in the presence of adequate glucose. Fajans *et al.*[10] have shown that there is no change in the urinary excretion of amino acids (urinary nitrogen) when DBI is given to fasted diabetic patients. Forbath and Clarke[11] stated that DBI does not increase intracellular glucose or glucose phosphorylation. These are essential steps in the initiation of anaerobic glycolysis. Even the relative blockade of Krebs cycle enzymes should induce hyperglycemia and not hypoglycemia.

The evidence for any definitive theory is yet not well delineated. The results thus far have been chiefly on animals, with dosage concentrations tremendously greater than those used in humans. Furthermore, they do not exclude other possible systems or mechanisms. Steiner and Williams[7] best stated the need for more thoughtful and objective evaluation as follows: "... the clinical trial is equally as important as the laboratory, so long as the overall benefits to the patient are objectively assessed in as many ways as possible."

Clinical Observations

It is evident that the biguanides are capable of lowering blood sugar levels in a wide range of diabetics including patients in whom other oral agents are ineffective. However, aside from the obvious gambit of "doing more than the other pills and doing it better," DBI shows unique ability to cooperate with insulin, and in a number of patients this drug has provided better regulation with fewer severe reactions and a subsequent more normal life. These were of that important minority, the unstable diabetic who is not doing well with even large doses of insulin, often given by multiple injection (TABLES 7 and 8).

DBI and its analogues are not effective in the successful management of human patients in the absence of exogenous or endogenous insulin. The juvenile-onset diabetics, with few exceptions, require supplemental, often smaller, amounts of insulin. Some cases were regulated with biguanide and diet alone for long periods but invariably required some insulin when their diabetes remission period terminated. Many adult-onset type diabetics can be regulated with diet and biguanide alone, but it is likely that some endogenous insulin is intact and in action.

Part of our earlier high side-effect incidence was due to the fact that the first attempts were to replace insulin completely. In the past year the goal has been to stabilize and regulate without primary regard for the size of the insulin dose. However, a high incidence of side effects is still possible, and until stabilization of dose is achieved in the more difficult diabetics, these patients must be carefully observed. In such cases the biguanides are a tactical rather than an operational weapon in the diabetic armamentarium.

In spite of the problems with the technique of regulation, no evidence of toxicity has yet been shown by liver, kidney, or hematological studies in several thousand patients. Some of these have been under observation for almost three years.

The incidence of severe hypoglycemic reactions in the well-regulated patient has markedly diminished with no hypoglycemic unconsciousness nor convulsive seizure in the entire study series (300 patients). There were many minor warning hypoglycemic episodes, however.

Further extensive experimental and clinical studies are needed. The analogue biguanides such as *n*-amyl (DBB) and *n*-butyl (DBV) appear to be better tolerated and are apparently superior to the phenethyl biguanide (DBI) in many patients. Joint administration of biguanide and sulfonylurea recently reported by Beaser[12] and Dolger[13] is based on the rationale that since the sulfonylurea drugs may stimulate the pancreas to produce insulin, and the

TABLE 7

Case No. 359 (H.D.) Age: 35 Duration diabetes: 22 years
Usual insulin: 90 units daily Diet: 2100 calories

	Hosp. day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Venous blood sugar values in mg. per 100 cc. (Somogyi-Nelson)													
Fasting		105	240	74	270	167	179	129	126	152	215*	39	34	101
11 A.M.		161	189	93	261	114	137	40	108	58	125	84	95	
3 P.M.	65		127	46	243	95	166	78	67	87	69	60		
Units insulin	90	60	64	67	67	56	43	60	42	48	48	50	44	40
DBB (mg.)						200	200	250	250	250	250	250	250	250

	Discharge medication			
	Breakfast	Lunch	Dinner	Bedtime
DBB mg.	100	50	50	50
Insulin units				
NPH	24	0	0	10
Regular	6	0	0	0

* Insufficient bedtime insulin given.

TABLE 8

CASE NO. 227: AGE, 10 YEARS, INSULIN 38 U. DURATION 2 YEARS, DIET-144-86-82

Hospital days	Capillary blood sugar			Insulin unit	DBI mg.
	A.M.	Noon	P.M.		
Insulin + weighed diet					
1	—	—	254 mg.%	38	
2	136	235	89	40	
3	75	—	—	37	
4	161	178	188	39	
5	108	197	116	40	
Weight diet + insulin + DBI					
6	92	—	45	28 +	150
7	155	70	55	20 +	150
8	—	54	35	16 +	150
9	124	74	62	8 +	100
10	97	59	—	8 +	100

biguanides appear to enhance or amplify insulin action, the combined action should be synergistic with smaller amounts of each. In our own series this effect has been demonstrated in several cases (TABLE 9).

There has been considerable statistical attrition in this combined series of adults and juveniles, as shown in FIGURE 2. Although 137 cases were classi-

TABLE 9

CASE NO. 221: AGE, 48 YEARS INSULIN 30 U. DURATION 8 YEARS WEIGHED DIET*

Hospital days	Blood sugar 7 A.M.	Blood sugar 11 A.M.	Blood sugar 3 P.M.	Insulin units	DBE gm.	DBI gm.
Insulin						
1	—	—	96 mg.%	30		
2	137	226	—	26		
3	88	159	109	30		
4	—	304	—	30		
DBE						
5	62	—	—		1.0	
6	76	100	—		1.0	
7	118	209	207		0.75	
DBE + DBI						
8	126	207	108		1.0	+ .150
9	114	—	95		0.5	+ .150
10	94	—	123		0.5	+ .150
11	71	110	124		0.5	+ .200
12	94	—	107		0.25	+ .200
13	102	141	103		0.25	+ .150

* In this case all blood sugar determinations are with the Somogyi-Nelson method. The 7 A.M. blood samples are venous, while the 11 A.M. and 3 P.M. samples are capillary. The DBI used was *n*-amylbiguanide; DBE represents chlorpropamide (Diabinese).

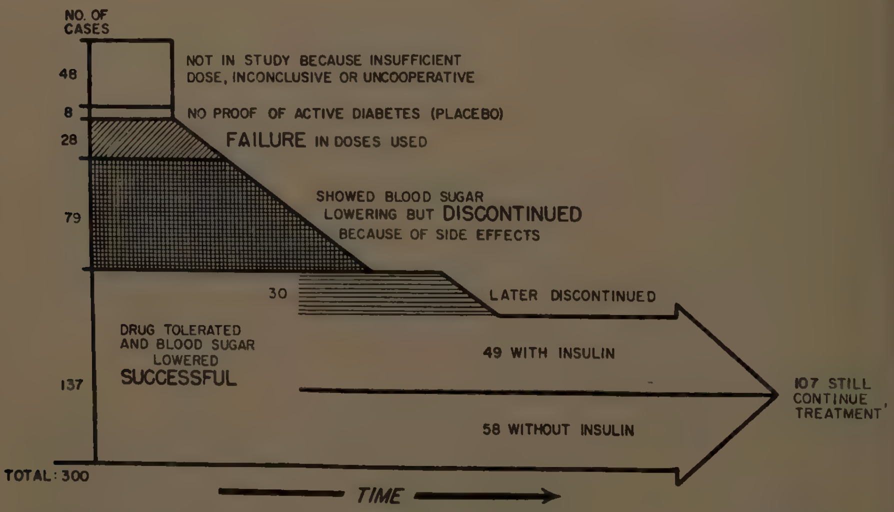
NO. OF CASES
48
NOT IN STUDY BECAUSE INSUFFICIENT DOSE, INCONCLUSIVE OR UNCOOPERATIVE
8
NO PROOF OF ACTIVE DIABETES (PLACEBO)
28
FAILURE IN DOSES USED
79
SHOWED BLOOD SUGAR LOWERING BUT DISCONTINUED BECAUSE OF SIDE EFFECTS
30
LATER DISCONTINUED
DRUG TOLERATED AND BLOOD SUGAR LOWERED SUCCESSFUL
137
49 WITH INSULIN
58 WITHOUT INSULIN
107 STILL CONTINUE TREATMENT
TOTAL: 300
TIME

FIGURE 2. Statistical attrition in 300 diabetics receiving biguanides (including severe and juvenile-onset cases).

fied as "successful," 30 of these were later discontinued from therapy for the following reasons: uncooperative, 12; unrelated complications, 6 (arterial graft, 1; chronic lymphocytic leukemia, 1; pregnancy, 1; coronary occlusion, 2; dead from myocardial infarction, 1). There were 12 other cases that were well regulated for varying periods of time but that were then discontinued because of later failure or because of a loss of well-being and tiredness that sometimes persisted in the face of normal blood sugar values.

Fifty-nine of the 300 study cases failed to respond to one of the sulfonylurea group either by trial or test. When given DBI or DBB, their response was: (A) successful, 34; (B) discontinued, 14; (C) failure, 5; and (D) deleted, 6.

Discussion

None of the presently available oral hypoglycemic agents is completely adequate, and all seem to nibble at the periphery of lower blood sugar values rather than strike at the basic aspects of diabetes. There is even some question as to whether or not the diabetes or the blood sugar level is being treated. On the other hand, contemporary forms of insulin sometimes have shortcomings, particularly in the therapy of the brittle, long-term diabetic. Many patients alternately fluctuate between quasiacidosis and convulsive hypoglycemia. Present types of insulin force the physician to commit himself 24 hours in advance, estimating the dietary intake and physical and metabolic activity as well as sudden onset of illness or accident. Insulin, however, is more physiological, and this fact has been proved over the years. Here, problems arise not so much from insulin per se as from the present forms of insulin.

There is no royal road to diabetes regulation and treatment and no shibboleth that is an easy pass-word to successful therapy. Diabetes is an individualistic problem, and no mass-produced treatment to date can solve all the facets. Each of the present components, diet, insulin, sulfonylureas and biguanides, is a tool to be used, singly or in combination, for a specific situation (FIGURE 3). These tools are of different weight and varying importance. The present age is one of specialization in all fields of endeavor. This is the era of the precise tool, in which careful techniques and special tools are used to achieve desired results. In diabetes these tools should be aimed at a more careful and precise regulation. As understanding increases and more tools are available, second-best and compromise therapy should be less tolerated than ever before. The era of the precise tool must not, for physicians, degenerate into an age of convenience, with an acceptance of lesser standards of treatment because of the convenience of oral therapy.

Summary

(1) A selected group of 244 patients including many difficult, unstable diabetics was treated for periods up to 28 months with the phenethyl (DBI) and *n*-amyl (DBB) biguanides.

(2) Eighty-eight per cent of this group responded with significant blood sugar lowering, while 44 per cent (32 per cent side effects, 12 per cent failure) of the total discontinued treatment because of side effects or failure to demonstrate blood sugar lowering in the doses used.

(3) At this time 107 still continue treatment from 3 to 28 months (average 16). Fifty-eight patients are currently receiving diet regulation and biguanides alone, while 49 others use supplemental but smaller amounts of insulin. Seventy-five of these cases have been observed for more than 1 year.

(4) While gastrointestinal side effects were present in a significant number of patients, they were reversed by lowering the dosage or discontinuing the drug.

(5) No evidence of toxicity occurred with serial tests of hepatic, renal and hematological function.

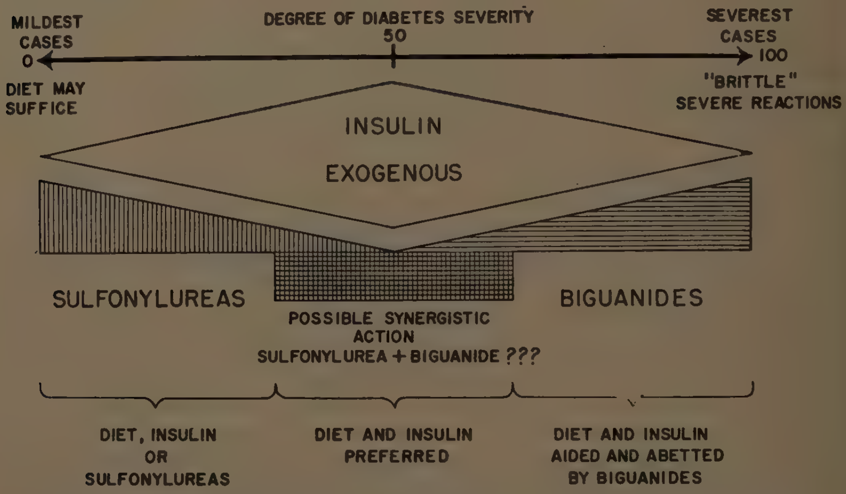
MILDEST CASES
DEGREE OF DIABETES SEVERITY
SEVEREST CASES
0
50
100
DIET MAY SUFFICE
"BRITTLE" SEVERE REACTIONS
INSULIN
EXOGENOUS
SULFONYLUREAS
BIGUANIDES
POSSIBLE SYNERGISTIC ACTION
SULFONYLUREA + BIGUANIDE ???
DIET, INSULIN OR SULFONYLUREAS
DIET AND INSULIN PREFERRED
DIET AND INSULIN AIDED AND ABETTED BY BIGUANIDES

FIGURE 3. Possible areas of optimal effectiveness of hypoglycemic agents. The scale depicting the degree of diabetic severity is a relative one. Exogenous insulin is effective in every type of diabetes. In the milder cases (recent onset, older adult), diet alone may suffice. The sulfonylureas also have been effective at this end of the scale. In the severest cases (unstable, juvenile onset), insulin is necessary, but some cases in this group are often difficult to manage and have frequent wide fluctuations and hypoglycemic reactions. Here the biguanides have in some cases demonstrated a stabilizing effect when used with smaller amounts of insulin. The effectiveness of joint sulfonylurea and biguanide therapy has been suggested but not adequately explored.

(6) Significant convulsive hypoglycemic reactions or resulting unconsciousness were not found.

(7) The exact mechanism of action is still a moot point, but probably the biguanides depend on exogenous or endogenous insulin for their effectiveness. No total juvenile-onset or severe, brittle diabetic has been successfully regulated without supplemental insulin for any considerable length of time.

(8) The biguanides, when carefully employed, in this series have been useful adjuncts in the stabilization of unstable and brittle diabetes.

(9) None of the oral agents is completely adequate. However, diet, the present forms of insulin, the sulfonylureas, and the biguanides are to be considered as tools of varying importance and utility, giving the physician a variety of therapeutic possibilities. This approach should encourage the more precise regulation of that highly individualistic metabolic state, diabetes.

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METAHEXAMIDE IN DIABETES THERAPY

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The search for the most potent and safest drug to be used in the oral treatment of diabetes has been continuous since the sulfonylurea drugs were first found to have hypoglycemic properties in 1942. Tolbutamide is the most widely used drug and its efficiency and safety has been attested by many reports.[1] It has been used in more than 400,000 patients with a prolonged satisfactory therapeutic response in more than one half of the cases and with minimal toxicity. In our own series of 200 patients, we obtained initial improvement in 84 per cent.[2] After 1 year of treatment this was reduced to 54.5 per cent,[3] and after 2 years of treatment, to 46.5 per cent.[4] This gives an over-all secondary failure rate of 38.5 per cent or about 3 per cent of those under treatment each month. This high rate of secondary failure is offset by a lower rate of primary failure than has been reported by others.[1] This difference is due to the fact that we continued to use the drug in 67 patients with initial poor or fair results. Our 46.5 per cent successful patients at the end of 2 years is comparable with the 53.4 per cent reported by Mehnert *et al.*[5] at the end of 20 months if all of their unselected patients are included as ours were.

Chlorpropamide was introduced because the same hypoglycemic effect can be obtained with a smaller dosage.[6] In our experience with 46 patients the comparable dose is about one third to one fourth that of tolbutamide, apparently due to a higher blood level resulting from the slower excretion. In general there is no significant difference in the results obtained with the two drugs. A few patients did show better results, but this can often be explained by the fact that it was easier for the patient to remember to take 2 or 3 tablets of chlorpropamide each morning than to try to remember to take 1 or 2 tablets of tolbutamide 2 to 4 times each day. When the drug is given in adequate doses any patient who does not respond well to one compound will not do well on the other. Since the dosage we used did not exceed 1 gm./day we did not encounter any patients with jaundice or other evidence of liver damage. There was one patient who had an aggravation of her anginal syndrome after being changed from tolbutamide to chlorpropamide, but this improved after the dose was reduced and the blood sugar allowed to rise.

Metahexamide has been used in 70 patients with excellent results in 31, good results in 14, fair results in 2, and poor results in 23 (only 51 patients are shown in FIGURE 1). There were 32 patients who had been treated with tolbutamide or chlorpropamide previously. Of the 14 with satisfactory results in the past there were 9 excellent, 4 good, and 1 fair response. Of the 18 with unsatisfactory results in the past, there were 14 failures, 2 poor, and 2 fair response. These last four patients either had been secondary failures or poor results previously. They have not been followed long enough for complete evaluation. It can be seen that these results are similar to those obtained with the other sulfonylurea drugs and that metahexamide will do nothing that tolbutamide will not do if an adequate dose is given.

We found that the private patients usually did better than the clinic patients. Much of this difference can be explained by the fact that the private patients were more careful about dietary control and the avoidance of obesity. The average maintenance dose for the private patients was 100 mg./day and for the clinic patients, 200 mg./day. The average for both groups was 150 with a range of 50 to 400 mg. There is probably no virtue in exceeding a dose of 200 mg. or, at the most, 300 mg./day. In those patients with a poor initial response the dose was raised to 400 and occasionally to 600 mg. before the drug was discontinued. In 21 patients who took 400 mg. or more for 1 to 30 days, there were 4 patients with features of acute gastritis characterized by epigastric hunger pains, nausea, and vomiting. These symptoms cleared promptly after the drug was discontinued. No liver function tests were conducted on the patients taking high doses. Alkaline phosphatase determination made on 20 patients on the usual maintenance dosage have shown no significant elevation.

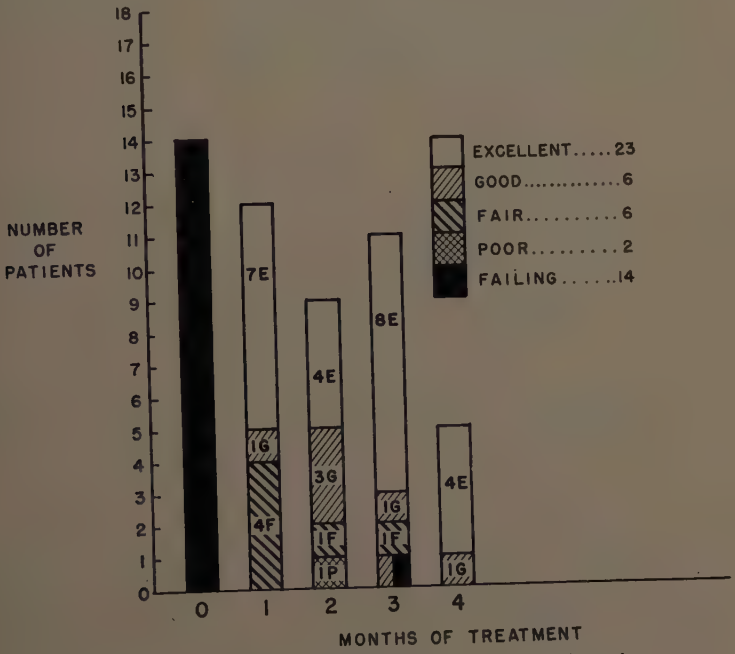

FIGURE 1. Results of metahexamide treatment in 51 patients.

One patient developed massive gastrointestinal hemorrhage after taking 200 mg. for a period of 2 weeks with excellent diabetic control. Complete gastrointestinal X-ray examinations were normal. Prothrombin time and blood clotting were normal. She had a similar episode 3 years ago with no apparent cause and there never have been any symptoms of gastrointestinal disease. Six pints of blood were required to raise the hematocrit from 22 to 42. She is now well controlled on 1 gm. of tolbutamide each day.

Another patient died of myocardial infarction after taking 100 mg./day for 2 days. There is no evidence that the drug was a factor in his death.

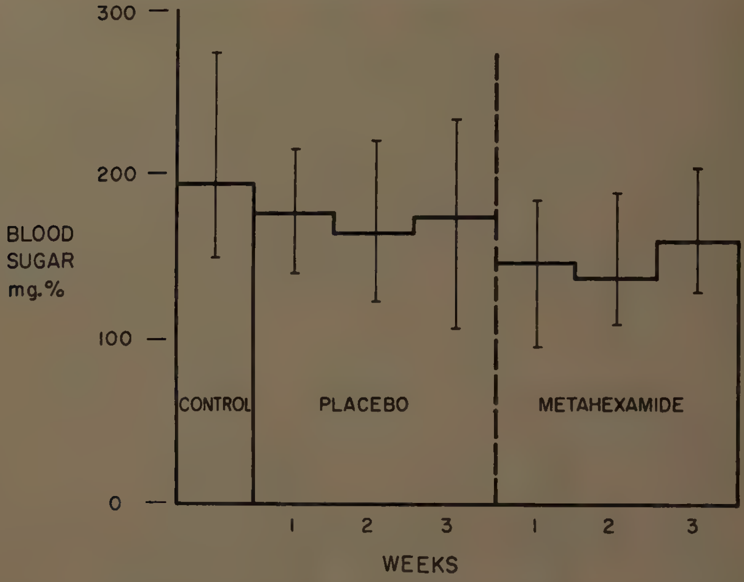

FIGURE 2. Comparative effect of placebo and metahexamide in eleven patients with a satisfactory response to metahexamide.

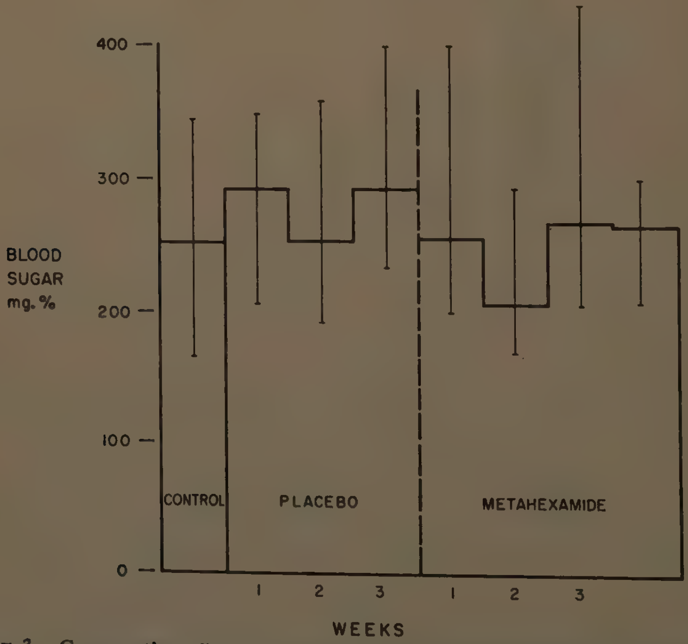

FIGURE 3. Comparative effect of placebo and metahexamide in seven patients with primary failure to metahexamide.

Twenty-one patients were given placebo tablets for 1 to 4 weeks before being placed on metahexamide. Almost all of these patients showed a fall in blood sugar during the first week or two of treatment followed by a rise as the novelty effect of the new medication wore off. This placebo effect explains some of the secondary failures that had been reported from other sulfonylurea drugs. FIGURES 2 and 3 show that the patients who obtained the best response to the placebo also obtained the best response to metahexamide. This can be explained by the fact that the patients with the more severe diabetes did not respond well to either. There were two patients who had been on 10 to 15 U. of insulin each day who were better controlled by the placebo than by metahexamide or tolbutamide. There have been others in whom the diabetes went out of control when the placebo was used, but who responded well to a sulfonylurea drug. Placebo studies are essential for the proper evaluation of any oral hypoglycemic drug and such studies should be done by the double-blind method.[7]

Summary and Conclusions

(1) The results obtained from metahexamide in a series of 70 patients are similar to those obtained from tolbutamide or chlorpropamide.

(2) Toxic effects of nausea, vomiting, and epigastric pain were seen in one fifth of the patients who took 400 mg. or more per day.

(3) The average maintenance dose is 150 mg./day and there is no value in exceeding a dose of 300 mg./day.

(4) Beneficial effects following placebo administration makes double-blind studies essential for the proper evaluation of any hypoglycemic drug.

(5) The only apparent advantage of metahexamide is the small size of the pill.

Addendum

After the submission of this paper for publication three patients who had been taking 100 mg. of metahexamide daily for more than 3 months developed jaundice. Two of these patients recovered promptly after the drug was discontinued, but the third died of hepatic necrosis about two weeks later. Because of the drug's serious hepatic toxicity even in low dosages, and because this toxicity is not readily apparent in the usual liver function tests, it is our opinion that further use of this drug should be discontinued.

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CLINICAL STUDIES WITH METAHEXAMIDE

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The investigation described below was conducted to confirm the antidiabetic properties of metahexamide, to study its therapeutic effectiveness, and to disclose the presence or absence of significant side effects. To this end, 35 adult patients with diabetes were placed on metahexamide* for periods as long as 4 months.

The study was done in the Diabetic Clinic, District of Columbia General Hospital, George Washington University Division, Washington, D. C., and the Medical Department of Prince George's General Hospital, Cheverly, Md.

Method of Study

Diabetic patients who were taking insulin, those inadequately controlled with tolbutamide or chlorpropamide, and patients only partly controlled by diet alone were studied. Some patients were hospitalized and others were observed in the outpatient clinics and in private practice. In the hospitalized patients, levels of sugar in the blood (fasting and post prandial) and in the urine (before meals and at bedtime) were determined. Ketosis, if present, was relieved by regular insulin injections and parenteral fluid. Later a measured diet was prescribed and control maintained by doses of regular insulin given fractionally according to urinalyses. The insulin dose ranged from 5 to 25 U., depending on the amount of glucose and acetone present. Metahexamide was then added to the regime, usually in a dose of 50 mg. three times daily before meals. If control with metahexamide was effective it was usually manifested in a few days by decrease in insulin requirement, reduction in blood and urine sugar levels, and by an increase in the feeling of well-being of the patient. If control was not attained at this dose level, metahexamide was increased by 50-mg. increments until a satisfactory response was noted. If metahexamide seemed ineffective after a 7-day trial, it was discontinued and insulin dosage adjusted for control.

Management with metahexamide was considered satisfactory if the average daily fasting blood sugar was substantially reduced and was in the region of 175 mg./100 ml. or below. A corresponding lowering of urinary glucose was required, the ultimate goal being complete aglycosuria. When at least 3 days' observation indicated these requirements had been attained, patients were given a supply of medication and discharged, to be followed in the clinic or office. Particular stress was laid on the need for frequent urine tests at home, and a written record of the results was requested. On the return visit, the patient was examined, home urine tests reviewed, and the fasting blood sugar and urinalysis for that day determined. If most of the urine tests were reported negative and if the blood sugar was 120 mg./100 ml. or less, the dose of metahexamide was reduced by 50 mg. If the blood sugar was between 120 and 175 mg., the original dose was maintained; if it was higher than 175 mg. and the urine tests showed considerable amounts of glucose, the dose was increased by 50-mg. increments until the blood and urine sugar levels indicated better control.

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* The metahexamide used in the study reported in this paper was supplied by C. J. O'Donovan of The Upjohn Company, Kalamazoo, Mich.

In a similar manner the patients not hospitalized were given the drug and requested to test the urine at home before every dose of metahexamide. Those taking insulin were given metahexamide concomitantly. Usually the dose of insulin was lowered by decrements of 10 or 15 U. as control was maintained by a constant dose of metahexamide, until eventually no insulin was needed or an optimum combination of insulin and metahexamide was obtained. If the blood and urine tests showed increasing amounts of glucose, metahexamide was stopped and insulin dosage adjusted until control again was apparent. Patients were seen at weekly intervals until management was considered satisfactory; thereafter the frequency of visits was decreased as improvement progressed.

TABLE 1

EFFECT OF METAHEXAMIDE ON INSULIN REQUIREMENTS

Patient	Age	Duration of diabetes (years)	Average insulin requirements		Average blood sugar		Dose of metahexamide (mg./day)
			Before	After	Before	After	
1	56	4	40	0	136	140	150
2	57	1	15	0	123	170	50
3	50	4	20	0	230	120	100
4	55	15	20	0	294	166	100
5	61	12	10	0	179	192	100
6	43	9	30	15	256	191	100
7	65	6	35	0	127	153	50
8	72	9	50	0	84	187	100
9	52	1/12	30	0	216	131	100
10	53	7	40	0	179	144	100
11*	39	4	10	0	142	144	100
12	76	2	10	0	242	178	100

* Previous secondary failure with tolbutamide.

Those patients taking tolbutamide or chlorpropamide, but with persistent glycosuria and fasting blood sugar always higher than 200 mg./100 ml., were considered not adequately managed. They were placed directly on metahexamide and followed in the same manner as described above.

Several patients were only partly controlled by diet alone. They were given metahexamide in the same starting dosage as described above, and the same type of follow-up was observed.

Results

The results of therapy with metahexamide in the 3 groups of patients are presented in TABLES 1, 2, and 3. A total of 35 patients have been followed for 1 to 4 months. Of these, 22 (63 per cent) showed adequate control either on metahexamide alone or in combination with insulin at a reduced dose.

FIGURE 1 illustrates the good response of a patient to metahexamide. He

TABLE 2

PATIENTS RESPONDING TO METAHEXAMIDE AFTER SECONDARY FAILURE WITH TOLBUTAMIDE OR CHLORPROPAMIDE

Patient	Age	Duration of diabetes (years)	Dose (mg. /day)		Average blood sugar		Dose of metahexamide (mg./day)
			Tolbutamide	Chlorpropamide	Before metahexamide	After metahexamide	
1	54	4	2000		240	193	200
2	58	8		500	228	108	200
3	62	9	1000		307	217	200
4*	30	1		750	236	178	100

* After 2 months control with metahexamide it was necessary to place this patient on insulin.

TABLE 3

PATIENTS SATISFACTORILY MANAGED WITH METAHEXAMIDE WHO WERE NOT PREVIOUSLY CONTROLLED WITH DIET ALONE

Patient	Age	Duration of diabetes (years)	Dose of metahexamide (mg./day)	Blood sugar under:		Duration of treatment (months)
				Diet	Metahexamide	
1	75	5	50	254	150	3
2	50	4	300	256	159	2
3	38	3	200	200	183	3
4	51	½	150	288	127	1
5	53	3	150	238	148	1
6	42	½	50	202	78	5
7	75	6	100	307	157	2

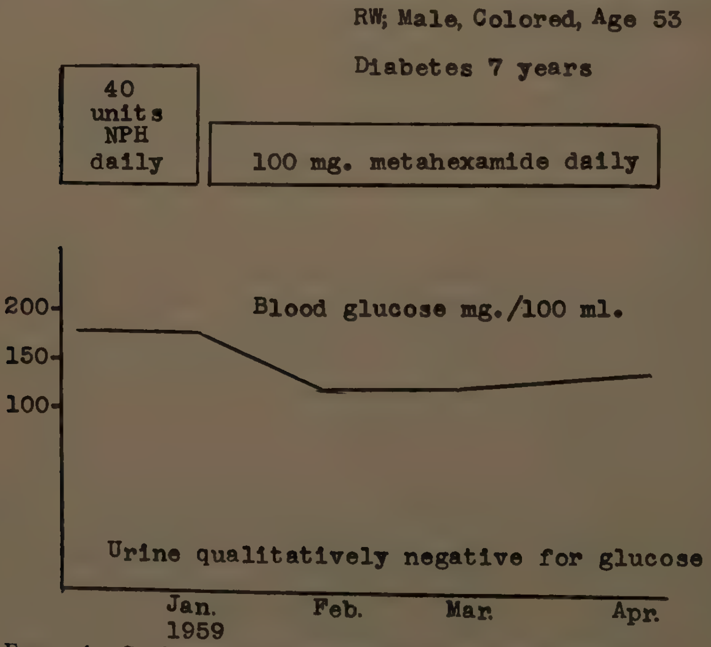

FIGURE 1. Good control with metahexamide after discontinuing insulin.

had been taking an average of 40 U. of insulin daily for 7 years. Transfer to metahexamide was accomplished smoothly and without incident. FIGURE 2 illustrates improved control with metahexamide in a patient not adequately managed with chlorpropamide.

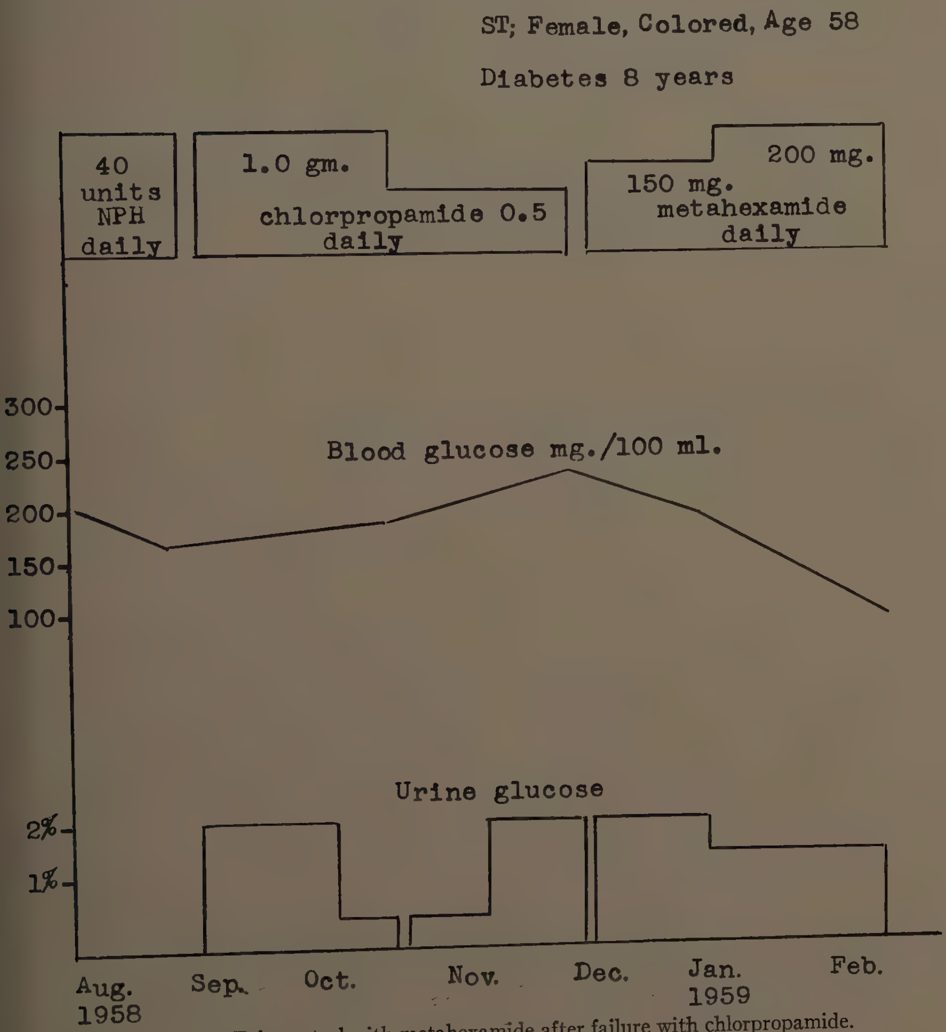

FIGURE 2. Fair control with metahexamide after failure with chlorpropamide.

A high degree of success (100 per cent of 7 cases) was noted with metahexamide given to patients only partly controlled by diet alone. The same good results with tolbutamide and chlorpropamide in this type of patient was reported previously by us.[1,2]

A summary of the effectiveness of metahexamide in the different classes of diabetics is shown in TABLE 4.

Toxicity

The usual precautionary laboratory studies (serial blood counts and urine examinations, and liver and kidney function tests) were made on hospitalized patients. With a daily dose range of 50 to 300 mg. metahexamide, no unusual bone marrow, liver, kidney, or thyroid effects have been noted. In fact, 1 case of acute cholangiolitic hepatitis complicating diabetes was adequately maintained on metahexamide after the diabetes was brought under control with insulin. The side effects noted (TABLE 5) were not severe. Only 1 patient noted hypoglycemic symptoms 10 days after starting therapy; these cleared promptly after eating. Two patients complained of epigastric burning, nausea, and vomiting. Generalized muscular weakness and vertigo were subjective complaints in 2 cases. One patient noted drowsiness and another itching without rash.

TABLE 4
RESULTS OF TREATMENT

Number of patients previously treated with insulin	23	
Satisfactory control with metahexamide alone	11	
Control improved by adding metahexamide	1	
Benefited by metahexamide	12	(52%)
Number of patients treated who had previous tolbutamide or chlorpropamide failure	5	
Satisfactory control with metahexamide	3	
Number of patients not controlled on diet alone	7	
Satisfactory control with metahexamide	7	(100%)
Total number of patients treated	35	
Satisfactory control with metahexamide	22	(63%)

TABLE 5
SIDE REACTIONS TO METAHEXAMIDE

	No. of patients*
Nausea and vomiting	2
Weakness	2
Dizziness	2
Itching	1
Drowsiness	1

* Of a total number of 35 treated.

Conclusions

In the patients studied, metahexamide gave effective control in a significant proportion of maturity-onset stable diabetics.

Metahexamide may be effective in some patients who are slipping out of control with tolbutamide or chlorpropamide.

Side effects of metahexamide in doses ranging from 50 to 300 mg. a day were minimal.

Acknowledgment

We are grateful to C. J. Burns and his laboratory staff for their assistance in performing laboratory toxicity studies.

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LABORATORY AND CLINICAL OBSERVATIONS WITH A NEW SULFONYLUREA

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This report describes our clinical and laboratory experience with 3-amino-4-methylbenzenesulfonylcyclohexylurea (metahexamide). This drug differs from other effective oral antidiabetic sulfonylureas in that this compound has been reported to combine therapeutic effectiveness with serum levels of long duration.[1]

Methods and Results

Reviewing the Boehringer Reports[1] on WP-40 (metahexamide) prior to our study, we observed that when the authors used single doses of 500 mg. and 1 gm. of metahexamide, they found half-value periods of 20 and 22.5 hours, respectively. We chose to use daily doses of 150 mg. of metahexamide, the recommended safe therapeutic dose,* in our studies. Half-value studies or the disappearance rate of metahexamide from the serum were studied, using the 150-mg. dose in order to relate these values to the previously mentioned 500 mg. and 1 gm. doses. Thirteen diabetic and 12 nondiabetic patients that had not previously received metahexamide were given, while fasting, a single oral dose of 150 mg. of the drug. Blood specimens were drawn prior to drug therapy and then 1, 2, 3, 5 7, 9, 12, and 24 hours after ingesting the metahexamide. Serum levels of metahexamide were determined using the method of Bratton and Marshall.[2] FIGURE 1 shows the number of hours after ingestion of the drug for appearance of peak serum levels of metahexamide. FIGURE 2 shows the number of hours required for the peak level of metahexamide in the serum to drop to the half-peak level. Although the range in the nondiabetic group is considerably less than that in the diabetic group, both groups showed wide variations. Our results appear to differ substantially from those reported by the Boehringer group.

FIGURE 3 represents the percentage of metahexamide remaining in the serum 19 hours after peak level of the drug. The choice of 19 hours after peak level was one of expediency. Percentages were calculated by dividing the serum concentration of metahexamide 19 hours after peak level by its concentration at peak level. It is apparent that the nondiabetics as a group demonstrate lower concentrations of serum metahexamide 19 hours after peak level than the diabetic group. The diabetic subjects seem to fall into 2 groups, 1 group showing 19-hour postpeak levels very similar to those of the controls, the other showing high levels of serum metahexamide. The parenthetical letters *G* and *P* indicate those patients who later, on continuous therapy, showed (*G*) good clinical control and (*P*) poor clinical control. It is obvious that no conclusion can be drawn from the single dose test in determining the value of this compound in the treatment of any given patient, with our limited number of patients. The striking difference in serum metahexamide levels after 19 hours within the diabetic group and the difference between the control group and certain of the diabetics may well warrant further investigation. The marked disparity of our results permit no conclusions to be drawn.

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* Recommended by C. J. O'Donovan, The Upjohn Company, Kalamazoo, Mich.

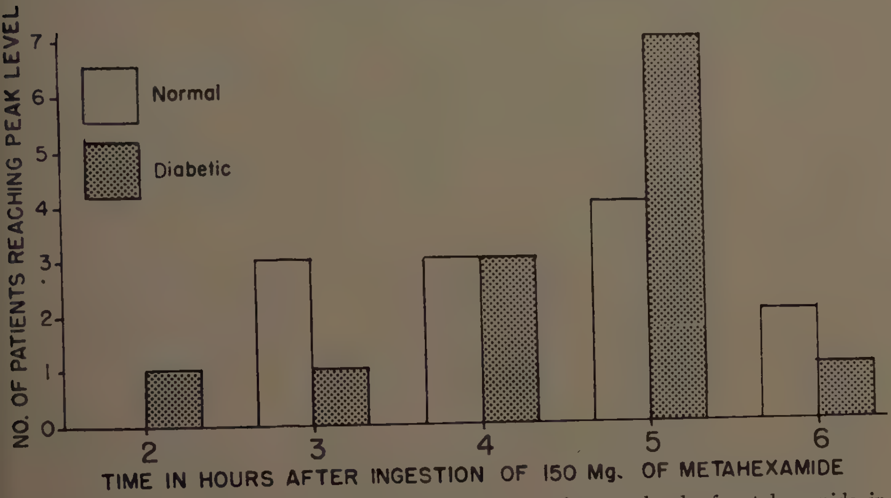

FIGURE 1. The distribution of appearance time for peak serum levels of metahexamide, in fasting diabetic and nondiabetic subjects, after the ingestion of 150 mg. of metahexamide.

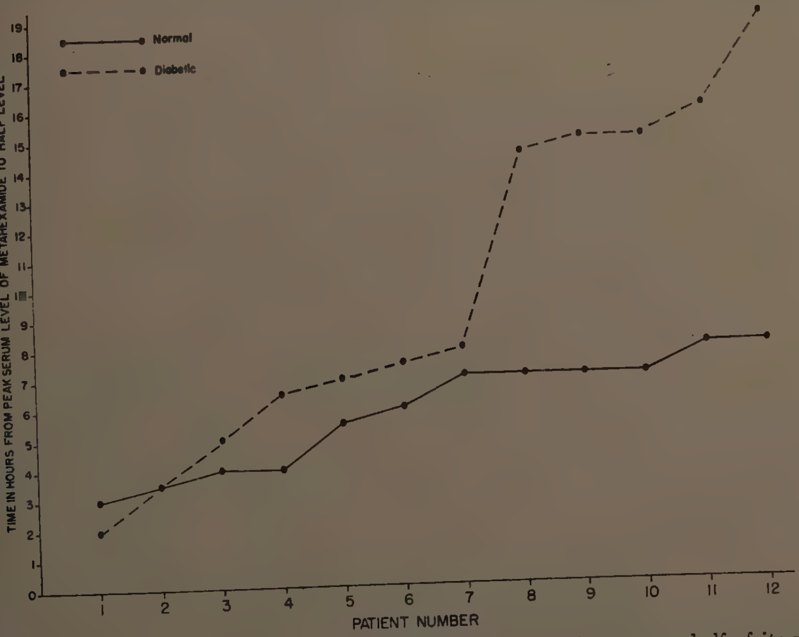

FIGURE 2. The time for the serum metahexamide level to drop to one half of its peak level in diabetic and nondiabetic subjects.

If metahexamide, as was previously stated, maintained serum levels of long duration, the possibility must be considered that patients on chronic therapy might increase their blood drug levels slowly. Serum metahexamide and blood sugar levels were frequently determined during the course of the chronic studies. Blood samples were always drawn at a given time in relation to the ingestion of the metahexamide. TABLE 1 shows a remarkable individual constancy of serum metahexamide level in 6 patients studied for periods up to 2½ months. TABLE 2 is interesting in that patient C. K. and several others in the initial single-dose test 19 hours after peak level had zero or negligible serum metahexamide levels. Continued therapy yielded detectable fasting levels. Patient C. K. (TABLE 2), after the single-dose test, was continued on daily doses of 150 mg. of metahexamide. After 1 month, serum metahexamide levels were determined before drug ingestion (150 mg.) and 5 hours later for 5 consecutive days. The constancy of the serum metahexamide level is most remarkable. It would appear that there is no cumulative effect of metahexamide with a constant daily dose.

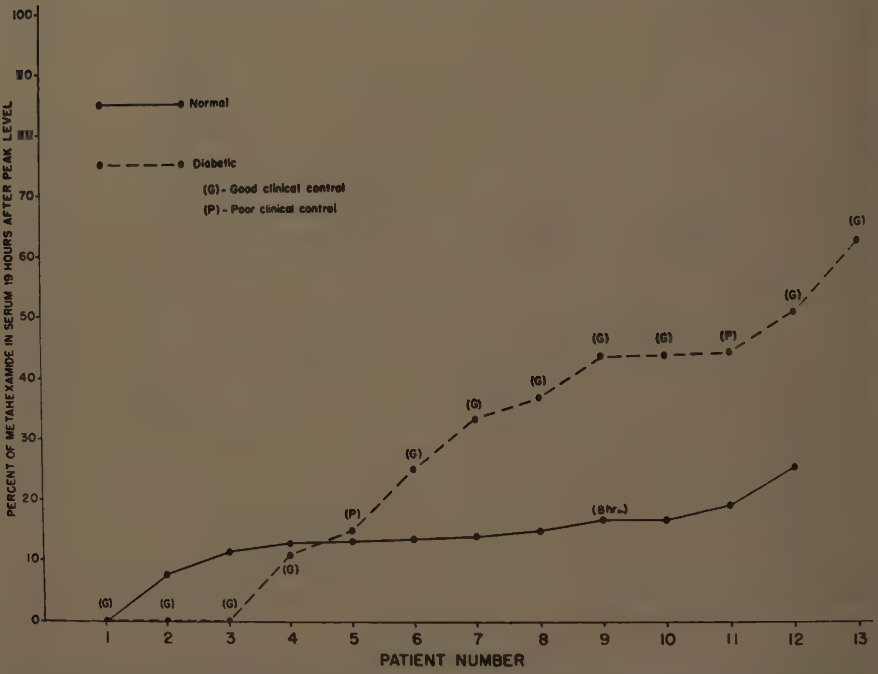

FIGURE 3. The percentage of maximum metahexamide in subject's serum 19 hours after peak serum level of the drug.

Sixty-seven diabetics were treated with metahexamide by oral administration. The initial treatment in all cases was 150 mg. given as a single fasting dose. Patients were selected chiefly on the basis of previous good response to tolbutamide. A few patients were selected because of the previous poor response to oral antidiabetic drugs and poor response to insulin, in the hope that they might benefit from this compound. Several patients on previous good control with insulin who requested a trial with an oral compound are also included. In all cases we began the study with a decrease in the chemical control by reducing the insulin or substituting a placebo for the previous oral compound until there was a rise in the blood sugar level and/or the appearance of glycosuria. Diet was unchanged from the pretreatment value.

TABLE 1

PATIENT CONSTANCY OF SERUM LEVELS OF METAHEXAMIDE

M. R.*,§		D. E.**,‡		I. N.*,‡		A. S.*,§		R. C.**,§		D. R.†,‡	
days	mg.%	days	mg.%	days	mg.%	days	mg.%	days	mg.%	days	mg.%
1	1.0	1	0.2	1	0.9	1	1.1	1	1.1	1	0.4
4	1.4	4	0.2	7	0.8	13	1.2	18	1.5	40	0.2
14	1.4	14	0.2	19	0.5	34	1.5	25	1.6	55	0.3
22	1.5	22	0.2	26	0.6	46	1.0	38	1.7	62	0.4
28	1.4	28	0.2	40	0.7	63	1.2	45	1.6	77	0.4
35	1.1	35	0.2	54	0.9			50	1.2		
42	1.1	42	0.2					61	1.4		
47	1.0							67	1.5		
64	1.2							82	1.2		
81	0.9										

Key: (*) daily dose of 150 mg.; (**) daily dose of 200 mg.; (†) daily dose of 100 mg.; (‡) daily predrug serum levels; and (§) serum drug levels 2 hours after drug ingestion.

TABLE 2

PATIENT C.K.: SERUM LEVELS OF METAHEXAMIDE

Single dose (150 mg.)*		Daily dose (150 mg)†		
Predrug (hours)	mg.% 0	Day	Predrug mg.%	5 hrs. later mg.%
1	0.5	1	0.5	1.6
2	0.6	2	0.5	1.5
3	0.5	3	0.8	1.7
5	0.4	4	0.5	1.6
7	0.3	5	0.5	1.7
12	0.2			
24	0			

* Prior to treatment.

† One month after treatment (daily dose 150 mg.)

Forty-five diabetics previously well controlled with tolbutamide were equally well controlled with metahexamide. They were transferred to metahexamide beginning with daily doses of 150 mg. In most cases this dose could soon be reduced. After several weeks of therapy, the amount of metahexamide employed was in the ratio of 5 to 10 mg./100 mg. tolbutamide. FIGURE 4 illustrates our results on H. L., a 50-year-old white male, a diabetic of 1-year duration and previously well controlled with diet and 1500 mg. tolbutamide daily. Within 24 hours after placebo therapy there was an acute rise in fasting blood sugar level and glycosuria. The patient was again controlled with tolbutamide, when he was abruptly transferred to metahexamide with no disturbance in control. After 10 days his dose was reduced to 100 mg; on the fiftieth day it was further reduced to 50 mg. daily, with which he continues to be well maintained.

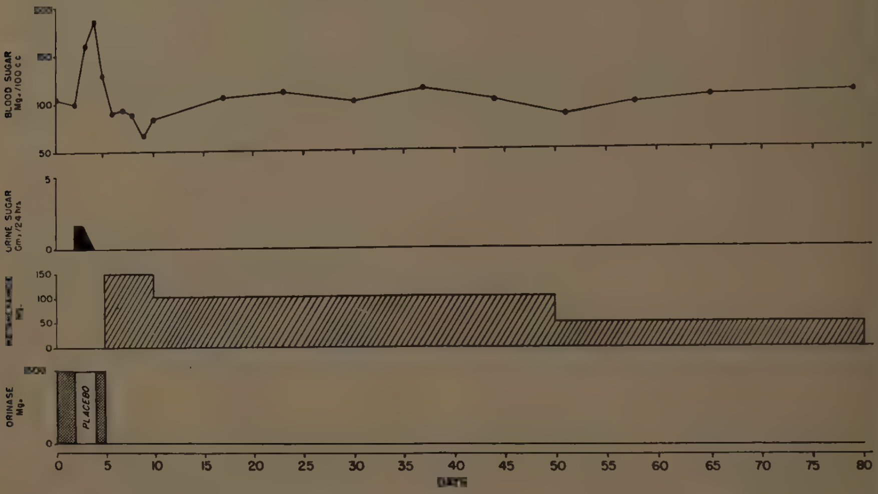

FIGURE 4. H. L., male, age 52 years, diabetic one-year duration. Previously well controlled with tolbutamide. Good control with metahexamide.

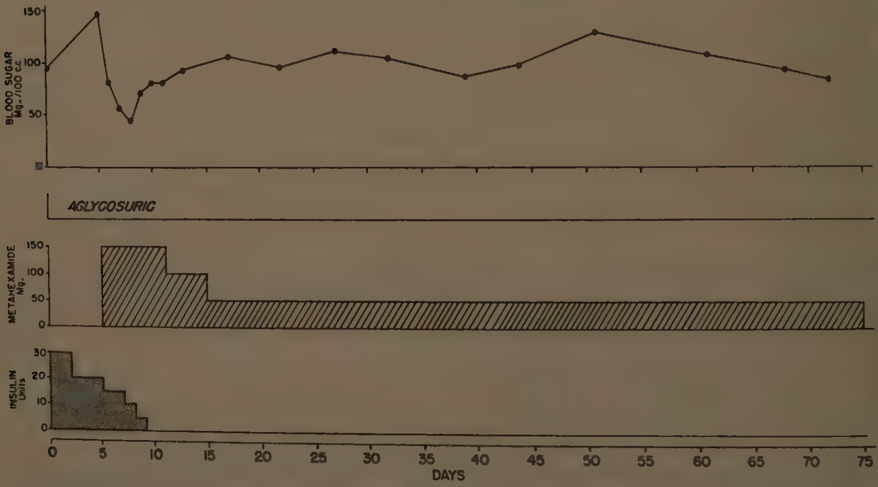

FIGURE 5. K. H., female, age 72 years, diabetes 5-years duration. Previously well controlled with insulin. Good control with metahexamide.

Eleven of 18 patients previously well controlled by insulin were transferred to metahexamide within 30 days or less. The remaining 7 of this group are still taking insulin in doses varying from 30 to 75 per cent of their intial amount. FIGURE 5 illustrates the transfer of K. H., a patient well controlled with diet and insulin, to metahexamide. This 72-year-old white female, a diabetic of 5-years duration, requested transfer to an oral agent because of difficulty in self administration of insulin. Her daily dose of insulin was first reduced to produce hyperglycemia and then increased until the fasting blood sugar was satisfactory. An initial dose of 150 mg. metahexamide was given and maintained until insulin was discontinued. Generally, we reduced insulin very slowly, rarely over 15 per cent in any given day. In this case insulin was reduced rapidly by our standards. The dose of metahexamide was gradually reduced until she is now maintained on 50 mg. daily in good control. FIGURE 6 shows the results with L. N., a 61-year-old white female, a diabetic of 12-years duration, transferred from insulin to metahexamide. This patient did not have good control with insulin, although we suspect she now follows her diet much better than prior to this transfer. Although her daily insulin requirement was 80 U., under close hospital supervision she leveled off at 50 U. The initial dose was 150 mg. of metahexamide and insulin was gradually reduced to zero. FIGURE 7 illustrates another patient, S. B., a 66-year-old white female, a diabetic of 16-years duration, previously maintained on diet and insulin, and now transferred to metahexamide. In this case we decided to try a greater percentage decrease in insulin. An abrupt rise in blood sugar necessitated an increase in insulin, 25 per cent above the initial dose. However, with slower decrease in insulin, a zero level was reached. FIGURE 8 illustrates patient S. P., a 41-year-old white male, a diabetic of 5-years duration, with sudden onset following corticosteroid therapy for penicillin reaction.

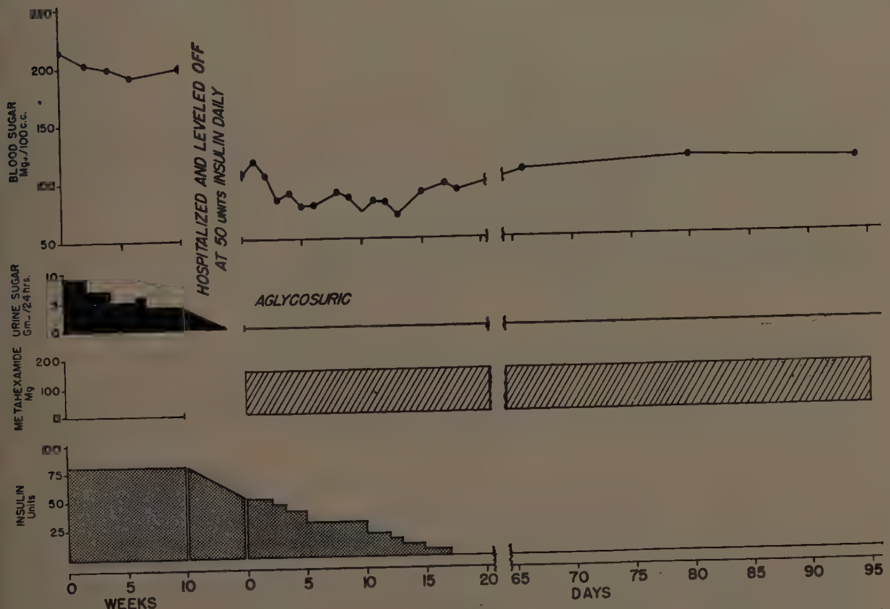

FIGURE 6. L. N., female, age 61 years, diabetes 12-years duration. Previous treatment insulin; well controlled with metahexamide.

This patient was not controlled with single or multiple doses of insulin, nor with the same dose of insulin plus tolbutamide, nor with phenethylbiguanide. The initial dose of metahexamide was 150 mg. plus 25 U. insulin a.c. breakfast and 10 U. insulin p.c. supper. The dose was increased to 200 mg. and later to

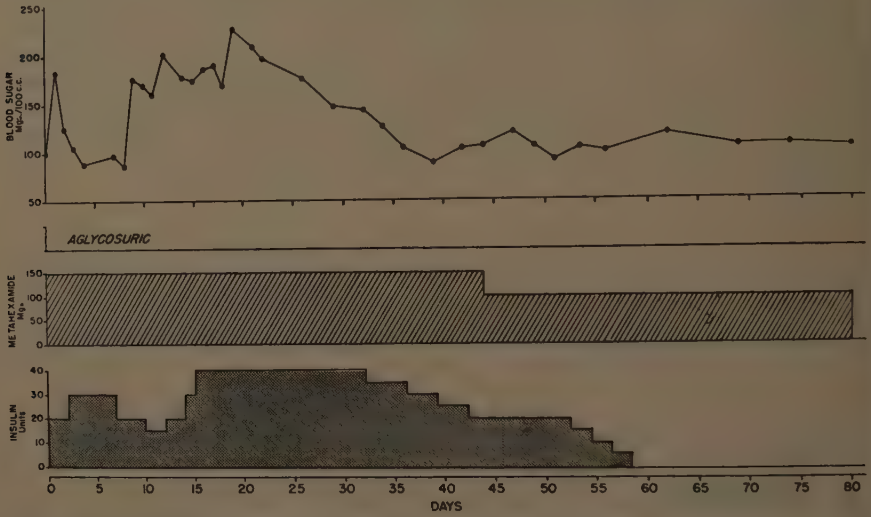

FIGURE 7. S. B., female, age 66 years, diabetes 16-years duration. Previously controlled with insulin. Good control with metahexamide

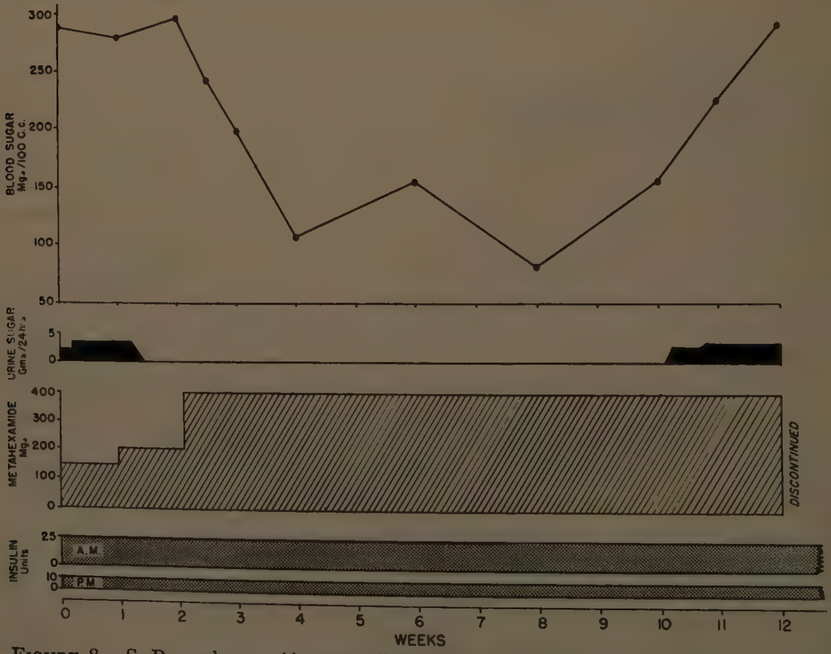

FIGURE 8. S. P., male, age 41 years, diabetes 5-years duration. Poorly controlled with insulin. Did not respond with metahexamide.

400 mg. daily. He showed evidence of control for a short period, then quickly reverted to the unstable state. Metahexamide was discontinued after 12 weeks.

Four of the 67 patients treated with metahexamide were taken off the drug, 1 for obvious failure, as stated above, and the remaining 3 because of side effects.

Toxicity

The following laboratory studies were done on all cases prior to and during therapy: serum transaminase, thymol turbidity, Bromsulphalein retention, alkaline phosphatase, blood urea, creatinine, urinalysis, phenolsulfonphthalein, complete blood count, and platelet count. Protein-bound iodine tests were done in most cases. All of these studies were repeated (except PBI) at weekly intervals for 4 weeks and then semimonthly up to 4 months.

Three of the 67 patients treated with metahexamide were removed from treatment with the drug because of side effects. One developed severe urticaria after 10 days of therapy. A second developed nausea after 3 days, which persisted for 2 weeks. Nausea disappeared with placebo and returned when the compound was again administered. The third patient developed severe vertigo when the drug was increased to 400 mg. daily. His symptoms disappeared with placebo but returned on daily doses of 50 mg. metahexamide. Laboratory tests were negative except in three patients who developed moderately elevated alkaline phosphatases. Two of these three patients had elevated transaminases. These patients show no clinical evidence of hepatic toxicity.

Summary

Clinical and laboratory data have been presented and discussed on sixty-seven diabetic patients treated with metahexamide for periods up to forty weeks.

Data have been presented showing: (1) the disappearance rate to one half the maximum serum drug level; (2) the amount of drug remaining in patients' sera 19 hours after peak level; and (3) the constancy of serum metahexamide levels in any given subject on constant daily doses (at constant time after ingestion of the drug), indicating absence of cumulative effect of metahexamide.

Our findings indicate that patients who responded well to tolbutamide therapy can be transferred uneventfully to metahexamide.

Acknowledgments

We thank the Upjohn Company, Kalamazoo, Mich., and Eli Lilly and Company, Indianapolis, Ind., for the metahexamide used in this study.

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BLOOD LEVELS FOR METAHEXAMIDE AND CAPILLARY VENOUS DIFFERENCES FOR GLUCOSE IN ELDERLY DIABETIC MEN USING INSULIN OR METAHEXAMIDE*

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A significant hypoglycemic action has not been demonstrated for the sulfonylureas in depancreatized man[1-7] or other mammals[7-9] nor has it been observed in completely alloxanized animals.[1, 9-13] This and other evidence[4, 14-20] support the hypothesis that an important action of the sulfonylureas is an increase in the production, release, or both, of insulin by the pancreas. On the other hand, in the fowl the peripheral utilization of glucose has been demonstrated in the absence of both the pancreas and the liver.[21, 22] Employing arteriovenous (A-V) glucose differences and the ratio of A-V glucose differences to arterial blood glucose (A-V/A), Madison and Unger[23] have demonstrated comparable uptakes of glucose by peripheral tissues in normal fasting dogs after the administration of insulin and tolbutamide. Their data "support, but do not prove, the thesis that one of the major physiologic effects of tolbutamide is the stimulation of endogeneous insulin secretion."

The experiments described in this paper were planned to compare the effects of insulin and metahexamide upon the peripheral utilization of glucose by measuring capillary venous (C-V) differences and by the claculation of the ratio of C-V glucose differences to capillary blood glucose (C-V/C).

Methods and Procedure

Five elderly (range 63 to 75 years, with an average of 66 years) ketoacidosis-resistant diabetic men, requiring from 10 to 40 U. of insulin daily (average, 26 U.) to maintain reasonable control were stabilized as to balanced diet and weight for at least 2 months prior to the study. A staisfactory transfer from insulin to metahexamide had been made 38, 29, 12, 93, and 55 days (average, 50 days) prior to the first test in cases 4, 7, 8, 10, and 11, respectively.† Until the day prior to the metahexamide tests (TABLE 1), 4 of the 5 subjects were receiving 50 mg. metahexamide orally as a single dose daily; the fifth (Case 8) was taking 100 mg. daily and had received the drug for only 12 days at a daily dose of 100 mg. On the morning of the testing, immediately following the taking of the zero-hour capillary and venous blood samples, each subject was given 500 mg. of metahexamide orally. Blood specimens were taken thereafter at hourly intervals for 5 hours while the patient fasted (TABLE 1). No further doses of metahexamide were used until completion of similar determinations in (1) patients given no medication (carried out 4 or 5 days after the metahexamide study) and (2) in patients administered insulin in the dosage employed by the individual before any treatment with metahexamide (carried out 3 days

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[*] The investigation reported in this paper was aided by funds from the Clinical Studies Division, Research Service, Veterans Administration, Washington, D. C., through a Veterans Administration Cooperative Diabetes Study.

[†] Case numbers of a previous report[24] have been retained.

TABLE 1

CAPILLARY-VENOUS (C-V) GLUCOSE DIFFERENCES IN DIABETIC MEN, DURING FASTING AND AFTER METAHEXAMIDE OR INSULIN

Medication	Case No.	Blood glucose (mg./100 ml.)																	
		Capillary						Venous						Differences					
		Hour						Hour											
		0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
None	4	80	98	110	102	98	108	77	90	98	95	90	90	3	8	12	7	8	18
	7	193	199	189	178	176	168	192	194	189	182	176	169	1	5	0	−4	0	−1
	8	124	121	124	128	123	113	120	117	117	123	117	113	4	4	7	5	6	0
	10	187	186	180	181	172	155	187	187	181	179	171	150	0	−1	−1	2	1	5
	11	130	133	122	115	100	108	120	122	113	112	94	102	10	11	9	3	6	6
Totals		714	737	725	704	669	652	696	710	698	691	648	624	18	27	27	13	21	28
Averages		143	147	145	141	134	130	139	142	140	138	130	125	3.6	5.4	5.4	2.6	4.2	5.6
Metahexamide (500 mg.)	4	108	99	101	79	71	80	77	78	76	60	63	66	31	21	25	19	8	14
	7	209	209	189	179	174	172	204	200	187	170	170	166	5	9	2	9	4	6
	8	100	75	72	70	72	90	90	72	70	60	66	66	10	3	2	10	6	4
	10	159	153	149	143	124	122	151	151	140	131	119	112	8	22	9	12	5	10
	11	130	122	115	108	93	92	103	103	94	93	90	78	27	19	21	15	3	14
Totals		706	658	626	579	534	536	625	604	567	514	508	488	81	54	59	65	26	48
Averages		141	132	125	116	107	107	125	121	113	103	102	97	16.2	11.0	11.8	13.0	5.2	9.6
Insulin 10 U.	4	88	72	51	50	41	41	86	66	44	51	41	43	2	6	7	−1	0	−2
40 U.	7	219	198	73	113	123	80	198	164	56	80	94	63	21	34	17	33	29	17
20 U.	8	173	156	81	57	56	57	160	133	62	37	35	43	13	23	19	20	21	14
40 U.	10	219	182	131	98	84	88	218	178	126	91	86	81	1	4	5	7	−2	7
20 U.	11	144	107	57	59	55	55	136	102	51	51	56	50	8	5	6	8	−1	5
Totals		843	715	393	377	359	321	798	643	339	310	312	280	45	72	54	67	47	41
Averages		169	143	79	75	72	64	160	129	68	62	62	56	9.0	14.4	10.8	13.4	9.4	8.2

following the determinations made during "fasting without medication"). Until all 3 test days were completed, regular insulin was used as necessary to maintain reasonable control of the diabetic state, but in no instance was this administered later than 15 hours before starting a series of blood determinations, nor was such supplemental insulin required frequently.

To minimize glycolysis, protein-free filtrates were prepared immediately after the withdrawal of the blood specimen. The determinations of glucose were made by the Somogyi-Nelson method.[25] From aliquots of the specimens removed for the estimation of glucose, the blood levels for metahexamide were determined by the method of Bratton and Marshall,[26] with this slight modification: before the addition of the coupling reagent, 1 cc. of concentrated hydrochloric acid was added to make the resulting dye soluble.

Results

C-V glucose differences and C-V/C ratios. Taking into consideration the age of our subjects, it may be seen from TABLE 1 that 2 of the 5 exhibited satisfactory blood glucose values at the beginning of the metahexamide tests, 2 showed only moderately elevated values, and 1 exhibited a high value. However, none showed more than 1+ glucose in the urine, and in no instance did more than 2 of the 4 daily fractional urines contain glucose. Metahexamide in maintenance dose had been continued until the day before the metahexamide test. On the morning of the metahexamide tests, the premedication C-V differences were greatest for those subjects who showed normal or nearly normal blood sugars, probably indicating a continuing activity of the metahexamide given the day before. Four or five days later, the fasting blood sugars were not greatly different from those observed at the end of the metahexamide regime. An attempt had been made to preserve about the same degree of control through the use of regular insulin when indicated by the appearance of more than 1+ glycosuria. During the interval between the determinations "without medication" and the test made following a single dose of insulin, (comparable to that used in the given individual before transference to metahexamide therapy), 2 of the patients showed a significantly higher fasting glucose level, while a slight elevation was present in 2 of the other 3 subjects. This occurred in spite of our efforts to maintain, by the use of regular insulin, approximately the same control as before.

As in A-V differences observed by other workers,[23, 27, 28] there was considerable variation in the C-V differences from patient to patient in the present studies. However, when averages were made and the standard deviations considered, it was noted that both insulin and metahexamide administration were associated with an increase in the C-V differences. We believe the higher premedication zero-hour C-V differences for metahexamide represented a continuing influence of maintenance doses used up to and including the morning before the test. In regard to the high zero-hour value for the insulin test, this average is comparable to that in the "without medication" test, if we exclude the 1 patient who had 2 doses of insulin on the preceding day. From the first through the third hours, it appears that the C-V differences were increased about equally by metahexamide and insulin in the dosages used. During this time, the fall of blood sugar was much more marked with insulin than with metahexamide (TABLE 1 and FIGURE 1). In all instances, however, patients receiving either metahexamide or insulin showed significantly greater decreases in blood sugar than were observed in patients who had simply fasted. C-V/C ratios are shown in FIGURE 2.

Metahexamide blood levels. Following a single dose of metahexamide, blood level determinations were made periodically for as long as 8 days in 13 mildly diabetic men and 8 nondiabetic subjects (TABLE 2). Sixteen sets of values were obtained in connection with a dose of 200 mg.; 5 with a dose of 500 mg.; and 2 with a dose of 800 mg. From TABLE 2 it can be seen that peak values were reached after 3 to 4 hours in all patients at each of the 3 levels of dosage. The higher the dose, the higher was the peak attained. There also seemed to be a direct correlation between the size of the dose and the time required for the blood level to reach half value. The half value was reached at the 800-mg. dose level somewhere between 24 and 48 hours; at the 500-mg. dose level between 20 and 24 hours; and at the 200-mg. dose, between 16 and 20 hours.

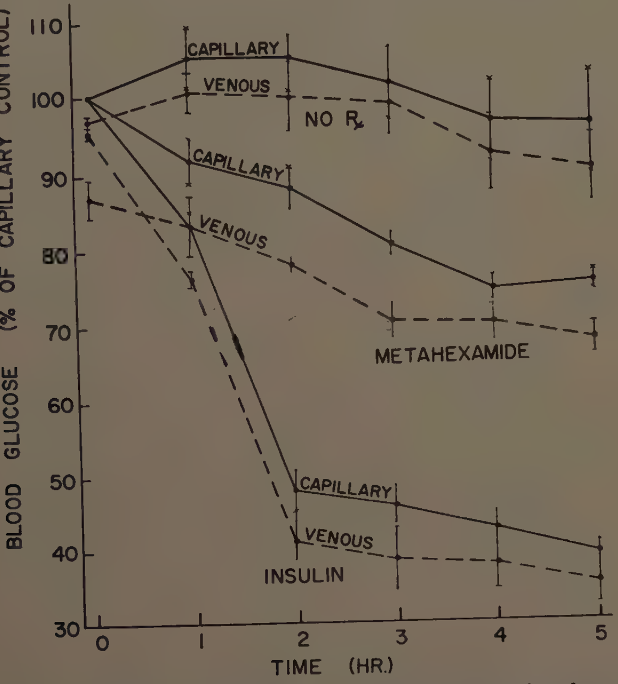

FIGURE 1. Blood levels for metahexamide and C-V differences for glucose in 5 elderly diabetic men using insulin or metahexamide. Each of the 6 curves shown is a composite of the data from the 5 subjects. For each pair of curves (capillary and venous) the capillary value has been arbitrarily assumed to be 100 per cent. The subsequent hourly capillary values and all of the venous values have been calculated for each patient in terms of this initial control. The curves themselves represent the averaged values of these percentages. The vertical bars at each hourly point represent standard deviations (SD). The differences following metahexamide and insulin as compared with no treatment have both been shown to be significant within the 5 per cent level of confidence except at 0, 4, and 5 hours.

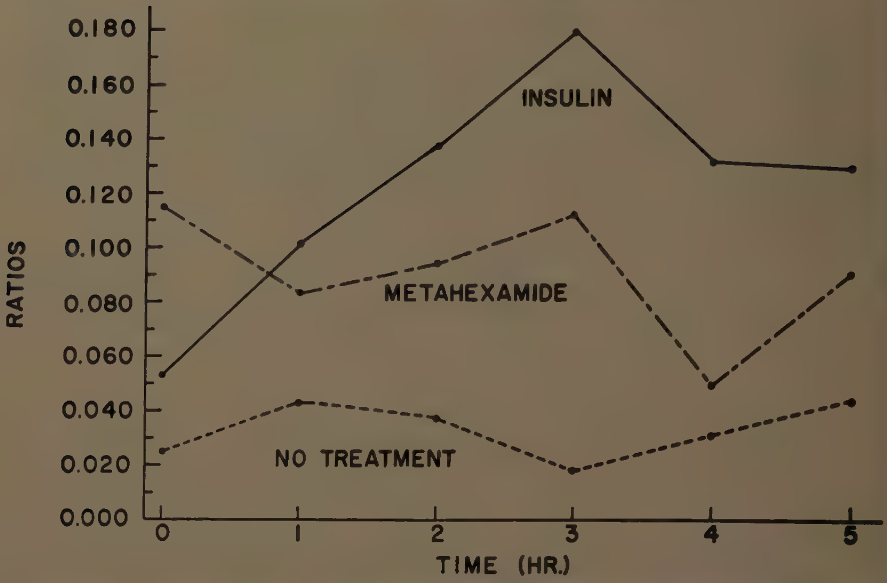

FIGURE 2. Blood levels for metahexamide and C-V differences for glucose in 5 elderly diabetic men using insulin or metahexamide. The curves have been calculated from the averaged values (in milligrams per 100 cc.) of the capillary and venous blood levels determined at the hours indicated. The ratios represent the difference between the averaged capillary and averaged venous blood values divided by the averaged capillary blood value. It is noteworthy that this ratio is highest during insulin administration, but still significantly higher during metahexamide treatment than during periods without medication. The high initial metahexamide ratio probably represents the effect of previous metahexamide therapy that, unfortunately, was not discontinued until 24 hours before data for this curve were obtained.

TABLE 2
METAHEXAMIDE BLOOD LEVELS IN 21 SUBJECTS AFTER ORAL ADMINISTRATION
mg./100 ml.

Hours	Dose (mg.)		
	800	500	200
1	1.5 (0.3–2.5)*	2.2 (0.78–2.64)*	—
2	3.0 (2.9–3.2)	2.7 (1.10–3.47)	1.7 (0.7–3.0)*
3	3.9 (3.3–4.3)	3.2 (1.82–3.74)	—
4	—	3.0 (1.28–3.61)	1.6 (0.8–2.9)
5	—	2.9 (0.99–3.53)	—
6	—	—	1.5 (0.8–2.9)
8	3.7 (3.3–4.0)	—	1.3 (0.7–2.7)
12	—	—	0.9 (0.5–1.1)
16	—	—	1.1 (0.6–1.2)
20	—	1.8 (0.82–2.72)	0.8 (0.6–1.0)
24	2.7 (2.4–2.9)	1.3 (0.42–1.89)	0.8 (0.3–2.1)
48	1.7 (1.6–1.9)	—	—
72	1.1 (1.0–1.3)	—	—
96	0.8 (0.7–0.9)	—	—
120	0.2	—	—
192		0.2 (0.0–0.5)	

* Figures in parentheses give the range of values.

Discussion

One acceptable parameter of peripheral glucose utilization has been the A-V glucose difference, although attention has been called to considerable variability in these values from subject to subject under the influence of insulin.[23,27,28] However, Madison and Unger have demonstrated that, in normal dogs, the responses of the individual animal to insulin and to tolbutamide are comparable. In other words, the animal that develops a striking hypoglycemia to insulin, a wide A-V difference, or both, will show a similar response to tolbutamide. This relationship did not exist for insulin and metahexamide under the conditions of our experiments in older, mildly diabetic men. Nevertheless, there seems to be little doubt that the peripheral utilization of glucose was furthered by metahexamide in these subjects. It is unlikely that these C-V differences alone accounted for the degree of hypoglycemia produced. The data are in accord with those of other workers;[23,29,30] namely, that in the presence of some pancreatic function or exogeneous insulin, a portion of the action of mtahexamide is concerned with the peripheral utilization of glucose. However, this does not account for the full effect.

Inasmuch as capillary blood is actually a mixture of arterial and venous blood, the changes observed become even more striking, as arterial-venous differences would probably have been greater. Moreover, the C-V differences obtained represented an uptake of glucose chiefly by the skin and subcutaneous tissues, whereas A-V differences reflect the activity of all skeletal tissues, including muscle.

That some workers[5,31-33] have failed to obtain data supporting evidence for the peripheral utilization of glucose may be due to differences in methods and experimental design. Many of these features have been dealt with at length by others.[23] Aside from A-V differences, other evidence for the peripheral activity of the sulfonylureas has been adduced. Ashmore and his associates[34] and W. L. Miller and his colleagues[10,35] have shown that under certain conditions tolbutamide will increase the formation of glycogen in muscle, although to not as marked a degree as insulin. Miller and his co-workers believe this action has been overlooked by a majority of observers because most of the experiments designed to prove or disprove this action have employed improper doses or insufficient periods of observation.

Because degradation of metahexamide occurs chiefly by hydrolysis and not by acetylation,[36] its removal from the blood is comparatively slow. This too may be the reason why the rate of removal of metahexamide is more dependent upon dosage than is the rate of removal of tolbutamide. Most observers give the half-time value as 20 to 22.5 hours, this figure being used irrespective of doses varying from 0.5[36] to 2.0 gm.[37] Our studies indicate dosage as an important factor in the half-value time of metahexamide. In view of the fact that disappearance of a single dose is rarely complete in any subject after less than 4 days, a cumulative action must be reckoned with in relation to continuing doses; this should be considered in any final adjustment of maintenance of the diabetic patient taking metahexamide.

Summary and Conclusion

C-V differences for glucose have been determined in 5 elderly diabetic men in the fasting state and after being administered insulin (26 U.) and metahexamide (500 mg.). There was no significant variation between the averaged differences obtained for metahexamide and for insulin, but both were significantly greater than the values obtained during fasting. In the doses used, the hypoglycemic action of insulin was greater than that of metahexamide.

Blood level half-value times determined for metahexamide showed that these varied directly with the size of the dose, but not in linear fashion.

Metahexamide is a potent hypoglycemic agent, a portion of the action of which is exerted, either directly or indirectly, upon the peripheral tissues.

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SUMMARY OF THE MONOGRAPH

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It is questionable whether the material presented in this monograph should be summarized at all, since this would of necessity provide a static ending to a dynamic and often scientifically discordant series of presentations. However, I shall attempt to integrate some of its highlights.

That a diabetic tendency can be inherited must be considered as definitely established. From Steinberg's review it appears that a recessive genetic trait is responsible. There is apparently no biological meaning to the earlier appearance of the overt disease in each succeeding generation. According to Remein, the prevalence of diabetes mellitus in the United States today appears to be approximately 19 per 1000 population, with only about one half of this number being recognized. This very fact underlines the continued need for developing ever more stringent criteria for what constitutes "normal" regulation of carbohydrate metabolism and improved methods of detecting decreased carbohydrate tolerance as a means of recognizing the disease earlier. This aspect of the problem is discussed by Fajans and Conn. It will be apparent that such research is necessarily of long range, for abnormal tests must be correlated with the eventual development of overt diabetes, and this may require many years. The pregnancy state presents a significant stress on the islet-cell system and thus affords a spontaneous test for any diabetic tendency as manifested by certain abnormalities in both the fetus and the newborn. Preliminary results from the long-range investigation by Wilkerson reveals that approximately 10 per cent of pregnant women may show an abnormal glucose tolerance test, that the same number may have at least one baby heavier than 9 lb. at birth, and that the same percentage gives a family history of diabetes. Since either insulin or tolbutamide treatment has been shown to return fetal mortality to normal, such investigations are of importance not only from the ecological point of view, but also in terms of prevention.

In addition to our concerted efforts to safeguard the survival of diabetics from their intrauterine existence onward, there is also an ever more thoughtful approach toward evaluating diabetics seeking employment. Generally acceptable definitions of the degree of control and permissible commitments have been devised and, according to Brandaleone, the diabetic is now generally accepted by industry as a reliable worker. The acceptance of such patients for life insurance is still rather limited, but Entmacher finds that more policies are being offered, although at least twice the normal premium is usually required. The principal reason for this lies in the accelerated vascular and neurological degenerative diseases that accompany diabetes, certain aspects of which have been discussed by Bloch and Ellenberg.

As diabetes is characterized most simply as an absolute or relative lack of insulin, islet-cell function is of crucial importance. Haist's broad discussion of the factors enhancing insulin production and secretion by the beta cells of the islets of Langerhans summarizes the field. For the growth of islet cells, anterior pituitary growth hormone appears to be essential. Insulin secretion is brought about by blood sugar elevation that may be caused by any of a number of agents. In contrast, sulfonylurea compounds stimulate insulin secretion in the presence of low blood sugar levels, at least in the growing rat. Prematurity-onset diabetes is accompanied by an absolute lack of insulin, whereas postmaturity-onset diabetes is usually characterized by normal insulin levels. In the latter type of the disease one is dealing with a subnormal islet cell response to a natural stimulus, namely, an elevated blood sugar level. There is failure of release of insulin, rather than of its synthesis, and it is in this group that the sulfonylureas appear to afford a beneficial therapeutic approach. Although the diabetic state is generally considered to be irreversible, one should not give up hope that, with a better understanding of the factors involved in islet-cell growth and insulin synthesis, at least partial reestablishment of the islet-cell function eventually may be achieved even in diabetics with insufficient islet-cell tissue. Development of new beta cells from ductal tissue in the rabbit given hyperglycemic agents is beautifully illustrated in the presentation of Volk and Lazarus. The electron-microscopic pictures of the beta cells shown by Lacy and Hartroft reveal the great variety of particulate organization in different species and should serve as the basis for a better understanding of the influence of various hormones on islet-cell function. Batts' ingenious use of changes in the Golgi apparatus as a means of demonstrating the secretory activity of the beta cells affords an elegant technique for further physiological study.

The fate of insulin, once released from the islets, has been investigated by the use of I^{131}-labeled insulin. The presentation of Unger and his associates clearly proves that insulin is bound by liver tissue in the dog and in man, and establishes a considerable degree of specificity for this binding. The interesting suggestion is made that very small amounts of insulin arising from the pancreas would be almost totally trapped by the liver and thereby exert essentially only a hepatic effect without affecting peripheral metabolism. The basic findings are well confirmed in the studies of Mortimore and Tietze in the rat, using a more direct approach of liver perfusion *in situ* and demonstrating considerable inactivation of the insulin by liver tissue. The one serious limitation of both of these studies lies in the use of insulin from other species and the changes in the insulin molecule brought about by I^{131}, however small the quantities used. Some of these facts were brought out by Berson and Yalow, who review the entire subject of insulin-binding antibodies in man. While everyone would unquestionably hail the advent of insulin as a milestone in diabetic management, the fact that every diabetic develops antibodies against insulin within two to three weeks reveals one of the potential defects in this type of therapy. These observations should encourage further work on the modification of the insulin molecule, research for synthetic insulinlike substances, and more studies on desensitization procedures by modern methodology. The theoretical advantage of the use of sulfonylureas in this respect are obvious.

The exact mechanism whereby insulin acts on the intermediary metabolism of the cell still remains a problem requiring much active work. For many years the rat diaphragm has served as a useful model of peripheral muscle tissue for the investigation of insulin action. The paper of Larner and his co-workers demonstrates the effective use of this technique in demonstrating glycogen synthesis independent of inorganic phosphate. Kipnis has introduced a special technique for removing the diaphragm that obviates cutting the tissue and thereby damaging it. Utilizing the classical *cut* preparation to measure phosphorylating capacity of muscle and the *intact* preparation, he shows that insulin affects primarily the rate of glucose transfer through the cell membrane, whereas adrenocortical hormones, pituitary growth hormone, and epinephrine decrease glucose tolerance by diminishing the rate of intracellular phosphorylation of glucose.

The studies by Park and Morgan and his associates on the effect of insulin and other agents on glucose utilization by the isolated rabbit heart, supplement the observations by Kipnis on the diaphragm of the rat. Including studies in both the aerobic and anaerobic state, these investigators show that the regulatory mechanisms for transport appear to be insulin and the level of aerobic metabolism, whereas the factors that regulate phosphorylation are the pituitary growth hormone and the adrenocortical secretions. The more anaerobic the environment, the faster both the penetration and the intracellular phosphorylation.

Developments in the glucose-transport theory of insulin action are succinctly summarized by Goldstein who, together with Rachmiel Levine, was a pioneer in this field. Goldstein's reference to a factor from exercising muscle that will act as a hypoglycemic agent much like insulin is of considerable interest. The work of Wick and Serif with 6-fluoroglucose strongly suggests the existence of more than one pathway for the penetration of glucose into the cell.

Spiro clearly demonstrates the relative independence of insulin for glucoseamine synthesis by the liver. This finding is somewhat surprising, since hepatic glucoseamine is a constituent of mucopolysaccharide and, as pointed out in discussion, the latter has been shown to be synthesized at a much diminished rate in the skin of the diabetic rat, explaining poor wound healing and, possibly, the small-vessel disease.

Cahill and his collaborators summarize much of their work on the effects of insulin on adipose tissue, now known to be very active metabolically, controlling glucose uptake, oxidation, and transformation into glycogen and triglyceride. The demonstrated blocking of the release of free fatty acids by insulin illustrates an important antiketogenic mechanism, and the effect on glycerol metabolism opens new vistas in intermediary metabolism.

The effect of insulin and tolbutamide on hepatic glucose release is covered in five papers: those of Steele, of de Bodo *et al.*, of Unger *et al.*, of Mahler *et al.*, and of Frawley *et al.* Utilizing various techniques, laboratory animals in different states of consciousness, and varying interpretations, two different concepts arise from this work: first, that small amounts of insulin reduce the secretion of glucose from the liver (Unger *et al.*, Reichard *et al.*, and Frawley *et al.*), and, second, that insulin has no effect on the hepatic output of glucose (Steele, Mahler *et al.*, and de Bodo *et al.*). The simultaneous use of arteriovenous differences in glucose across the hepatic bed and glucose-C^{14} dilution studies might some day resolve the differing points of view.

Following these basic discussions, the final section of this monograph is taken up by more clinical presentations. I cannot undertake detailed discussion of these papers here. Suffice it to say that tolbutamide appears to be by far the safest and least toxic of the oral hypoglycemic agents. Chlorpropamide appears to be 4 to 6 times more potent, and this enhancement is almost entirely due to its longer active half life in the tissue fluids. This characteristic, of course, has both advantages (convenience) and dangers (hypoglycemia) attached to it. The effectiveness of this drug in cases refractory to tolbutamide is of doubtful significance, since in some reported cases the potency differential may have been responsible rather than the different nature of the drug. The acceptance of the beta-cytotropic theory of action of the sulfonylureas limits their use to postmaturity-onset cases; this is not the case with the biguanides. However, the latter drugs appear to have a somewhat limited usefulness because of their tendency to give rise to gastrointestinal upsets as the dosage is increased. Used jointly with insulin in "brittle" diabetics, there is a definite indication in selected cases.

Metahexamide received considerable discussion by a variety of authors, but in both the papers and the discussions an undertone of apprehension was noticeable lest the early indications of toxicity, including hepatic necrosis, might eventually limit the usefulness of this new agent. Subsequent to the conference upon which this monograph is based the manufacturers of this compound withdrew it from experimental trial because of the rapidly mounting number of serious and, in some instances, fatal liver involvement.

There are thus, at the time of this summary, four therapeutic agents available in the therapy of the various types of diabetes. Insulin is still the mainstay for diabetic control in the prematurity-onset type and is used to advantage in many middle-aged patients, especially those who are underweight. Tolbutamide is the safest and most widely used oral hypoglycemic agent for control of patients with postmaturity-onset diabetes, using less than 40 units of insulin for less than 10 years and, preferably, without a history of coma. The same indications apply to the use of chlorpropamide, with its definitely longer action but also higher incidence of toxicity. Finally, the biguanides, notably phenethylbiguanide, have a somewhat limited application in mild diabetics or in conjunction with insulin. These compounds differ from the sulfonylureas by showing some activity in the prematurity-onset type of diabetes as well.

To take advantage of this greater choice of hypoglycemic agents, the clinician needs an ever more comprehensive understanding of basic physiological processes and of the changes brought about by the diabetic state. By bringing together basic and practical considerations, a publication such as this will, we believe, benefit a large number of laboratory workers and clinicians alike.

Printed in United States of America

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